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Review

Role of H2AX in DNA damage response and human cancers

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ABSTRACT

H2AX, the evolutionarily conserved variant of histone H2A, has been identified as one of the key histones to undergo various post-translational modifications in response to DNA double-strand breaks (DSBs). By virtue of these modifications, that include acetylation, phosphorylation and ubiquitination, H2AX marks the damaged DNA double helix, facilitating local recruitment and retention of DNA repair and chromatin remodeling factors to restore genomic integrity. These modifications are essential for effective DSB repair, so is their removal for cell, to recover from checkpoint arrest. Because of these vital roles during DSB signaling and also its activation during early cancer stages, H2AX is emerging as an intriguing gene in tumor biology, supported further by frequent deletion of the region harboring this gene. This review focuses on the insights gained from recent studies on dynamic regulation of H2AX in DSB repair. Also, posing future challenges in the area of chromatin reorganization and retention of epigenetic signature post-DSB-repair with implication of its haploinsufficiency in human cancers.

Contents

1. Introduction

DNA repair is the cellular process aimed at the correction of DNA damage before it is fixed as a mutation or chromosomal aberration, leading ultimately to cell death, tumorigenesis or aberrant germ cell formation. Where on one hand, the long-term survival of the species may be enhanced by genetic changes, the survival of the individual demands genetic stability. All prokaryotic and eukaryotic cells therefore possess highly efficient and specific repair mechanisms to contend with the various types of DNA damage. One important form of DNA damage is the double-strand break (DSB), where the two complementary strands of the DNA double helix are broken simultaneously at sites sufficiently close to one another such that base pairing and chromatin structure are insufficiently strong to keep the two DNA ends juxtaposed for a longer period of time [1]. DSBs arise by an exposure to ionizing radiation and radiomimetic drugs or as a result of replication fork collapse or through the action of reactive oxygen species produced as a by-product of normal metabolic processes. DSBs are also generated as part of normal cellular processes, such as V(D)J recombination in developing lymphocytes, meiotic recombination in germ cells and mating-locus type switching in yeast [2–7]. Even a single DSB proves detrimental if left unrepaired or when misrepaired [8]. The outcome of DSBs not efficiently taken care of is

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also evidenced by the phenotypes of genetic disorders involving faulty DSB repair and characterized by predisposition to cancer, such as Bloom syndrome (BS), Werner syndrome (WS), Nijmegen Breakage syndrome (NBS), Fanconi anemia (FA), Ataxia telangiectasia (AT) and Ataxia telangiectasia like disorder (ATLD) [9,10].

DSB repair process in eukaryotes takes place in the context of chromatin structure and past decade has unraveled the modifications of chromatin itself as an early response to DNA damage [11,12]. One of the best-characterized chromatin modification events in DNA damage response is the phosphorylation of histone H2AX, referred to as γ-H2AX. This phosphorylation event at serine 139 of histone H2AX in mammals and serine 129 in H2A of yeast, is one of the earliest responses to DNA DSBs playing a critical role in their recognition, signaling and repair [13]. In the past decade, the importance of several post-translation modifications (PTMs) including acetylation, methylation and ubiquitination, involved in signaling of DNA damage as well as in facilitating actual repair of lesions, has become evident [14]. However, these histone modifications and their dynamics have been studied extensively in the context of DSB repair [15–17]. This review focuses on the recent advancements in understanding the role of histone H2AX in the recruitment of DSB repair factors, histone-modifying enzymes, ATP-dependent nucleosome remodeling complexes and cohesin complexes, to the sites of DNA damage and the regulation of H2AX activity by phosphorylation and ubiquitination with an emphasis on mammalian system. The review also deals with the involvement of H2AX in human cancer and its role as a tumor suppressor.

2. Post-translational modifications of histone H2AX

2.1. Histone H2AX phosphorylation and recruitment of repair factors

Among the four major core histones that make up the nucleosome, H2A is known to have the largest number of variants [18]. Two H2A variants, H2AX and H2AZ are constitutively expressed and distributed throughout the genome. H2AX represents between 2 and 25% of the total cellular H2A, whereas H2AZ accounts for ∼10% [18]. H2AX variant contains a conserved SQ(E/D) motif in the extended C-terminal tail that is known to be phosphorylated at a serine residue in response to DNA damage [13]. Both H2AX and H2AZ are important for DNA damage responses (DDR) [18] but in contrast to H2AX, which is profusely modified in response to DDR, no post-translational modification has been reported for H2AZ. In lower eukaryotes, such as yeast, the conserved SQ motif present in the (non-extended) H2A proteins, and in H2Av, a variant of H2AZ in *Drosophila*, also show the property of phosphorylation at this motif in response to DNA damage as observed in mammalian system [19]. For convenience we refer to H2A of yeast as H2AX in this review.

H2AX is known to be phosphorylated by members of phosphoinositide 3-kinase related protein kinases (PIKKs) such as ATM and Rad3-related (ATR) (Tel1/Mec1 in yeast), or DNA-dependent protein kinase (DNA-PKcs) in response to genomic insult [20], although it has been recently known to occur outside the DNA damage response (DDR) as well [21]. Whereas ATM is a dominant physiological mediator of H2AX phosphorylation in response to DSB formation, ATR phosphorylates H2AX in response to single-strand breaks and during replication stress. In contrast, DNA-PKcs is known to mediate H2AX phosphorylation in hypertonically stressed cells as well as in response to DNA fragmentation during apoptosis [22,23]. However, in response to DNA damage caused by ionizing radiation, all these kinases may function redundantly to phosphorylate H2AX with half maximal amounts at 1–3 min [20]. Upon DSB induction, the use of an antibody specific to γ-H2AX has facilitated its visualization as discrete nuclear foci in mammalian cells; each focus corresponding to one DSB [24–27]. Studies using laser scissor as a source of DSBs have revealed a concurrent pattern of γ-H2AX staining along its path [27]. H2AX has recently been shown to get phosphorylated independent of DSB also, e.g., in response to UV-irradiation and hydroxyurea treatment. However instead of discrete nuclear foci formation, UV induces diffuse pan nuclear staining of γ-H2AX throughout the cell cycle and the strongest is observed at the S-phase. It has further been observed that the G1 expression of UV-induced γ-H2AX was dependent on nucleotide excision repair (NER) factors [28,29]. While UV-induced phosphorylation of H2AX has not yet been characterized completely, DSB-induced γ-H2AX formation has been extensively studied. In response to DSB it is now known that γ-H2AX extends up to 50 kb on either side of the DSB in yeast [30–32] and up to 1 megabase in mammalian cells [18,33]. In this context it would be interesting to know whether H2AX is uniformly or randomly distributed in the genome. Given that it comprises only upto 25% of the H2A pool, not all nucleosomes will have equal quantity of H2AX molecules. How DSBs induced in such H2AX deficit regions are taken care of, remains a question? Are DSBs generated randomly in response to genomic insult or does the genome architecture, e.g., the abundance of H2AX and many other factors, make certain regions refractory to damage and others more susceptible.

Fig. 1 summarizes the DSB-induced signaling cascade and chromatin remodeling initiated by phosphorylated H2AX following ATM activation, which is discussed sequentially in this review. The key component of the DSB-induced H2AX phosphorylation, i.e., the ATM protein kinase is known to get activated through an autophosphorylation site on serine1981, which leads to dissociation of the inactive ATM dimer into single protein molecules with kinase activity [34–36]. A tri-protein complex called as MRN complex (Mre11-Rad50-Nbs1) recognizes DNA damage, recruits ATM to the site of damage and also functions in targeting ATM to initiate phosphorylation of the respective substrates (Fig. 1a and b) [37–39]. Other than DSB-induced activation, changes in higher order chromatin structure, resulting due to relaxation of topological stress following DNA breakage, is also known to activate ATM [36]. However, the mechanism of ATM activation seems to be more complex as revealed by recent studies which suggest that activation also requires prior ATM acetylation, mediated by Tip60 histone acetyltransferase [40]. Apart from H2AX, the early downstream target substrates phosphorylated by ATM includes, H2AX, BRCA1, 53BP1 and MDC1 [35,41]. In response to ionizing radiation, these repair proteins including the MRN complex and Rad51 have been shown to colocalize with γ-H2AX at intranuclear sites called 'ionizing radiation induced foci' (IRIF) [42–46]. MDC1 (mediator of checkpoint signaling 1) interacts directly with γ-H2AX via its BRCT domain and [47] further amplifies the H2AX phosphorylation signal either by tethering ATM thereby recruiting more ATM molecules or by preventing H2AX dephosphorylation [48]. This in turn results in accumulation of more and more repair factors to the site of damage including BRCA1 and the checkpoint adaptor protein, p53-binding protein 1 (53BP1) [49]. However, the wave of histone ubiquitination following H2AX phosphorylation explains the actual mode of BRCA1 recruitment, which is discussed later in a separate section. Phosphorylated histone H2AX has also been well-documented as a signal for the recruitment and efficient accumulation of 53BP1 at sites of DSBs [32,47,50,51]. Much evidence have been obtained from studies in yeast where H2AX S129 phosphorylation and H3 K79 dimethylation in *S. cerevisiae* contribute to recruitment of Rad9 (53BP1) to chromatin and establish G1 checkpoint arrest [52,53]. It is proposed that phosphorylation of H2AX alters the chromatin configuration exposing the methylated H3/H4 lysine residues which is then