Phosphorylation of histone H2A.X as a DNA-associated biomarker (Review)

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Abstract. The complex genomic structure of eukaryotic cells is primarily achieved by the binding of DNA to histones. Different members of the histone families form a complex with genomic DNA and, as a nucleosome, constitute the functional unit of chromatin. In addition to their structural functionality, histones are also involved in other molecular mechanisms, such as DNA damage recognition and repair. A very important factor of DNA damage management is the histone H2A.X. The phosphorylation of H2A.X initiates various processes of the DNA repair systems and plays significant roles in cellular regulation. The H2A.X phosphorylation status represents a central sum parameter for genome integrity and allows conclusions to be drawn about DNA-associated processes in cells and tissues. As a biomarker for DNA damage and genotoxicity, as well as a clinical marker for radiotherapy outcome, drug efficacy and tissue regeneration, the H2A.X phosphorylation status represents an effective biomarker for current and future biomedical applications. The present brief review article provides an overview of the various molecular functions and cellular events in which the phosphorylation of histone H2A.X can occur.

Contents

1. Introduction

In eukaryotic cells, genomic DNA is present as chromatin. This complex structuring is achieved as the DNA is bound to DNA-binding proteins, the histones. The complex of DNA and histones is known as a nucleosome and represents the functional unit of chromatin (1). Such a nucleosome includes a DNA region of 145-147 base pairs, which is wound as a superhelix around a histone octamer. The histone group consists of four families: H2A, H2B, H3 and H4. Two histones from each family form a dimer, H2A-H2B and H3-H4, which finally assemble as two tetramers $(H2A\text{-}H2B)_2$ and $(H3\text{-}H4)_2$ to form the functional histone octamer (1,2). The histones of each of the four families are encoded by several genes and are expressed during DNA synthesis in the S phase of the cell cycle (3).

2. Histone H2A.X and its phosphorylation

The histone family H2A consists of the members, H2A.1, H2A.2, H2A.X and H2A.Z (4). The main part of the histone family is represented by H2A.1 and H2A.2. The two isoforms differ in only a few amino acids and thus far, no differential functions have been detected. In eukaryotes, H2A.Z comprises ~10% of the H2A histones, with H2A.X comprising even up to 25%. In mammals, however, H2A.X is much less expressed and represents only up to 10% of the H2A histones (5).

H2A.X is 124 amino acids in length and differs from the other members of the H2A family by a highly conserved 22 amino acid domain at the C-terminus. The C-terminal motif Ser_{139}-Gln_{140}-Glu_{141}-Tyr_{142} is used for post-translational modification of the histone, where the protein can be phosphorylated at Ser_{139} (5,6).

H2A.X, as with all other histones, serves to structure and stabilize the DNA (7). It also has a very specific function in the complex DNA damage detection and repair machinery of higher eukaryotes. The phosphorylation of H2A.X at position Ser_{139} (γH2A.X) is one of the first signals for the detection of DNA double-strand breaks (DSBs) and an essential step for the initialization of DNA repair (8,9). DSBs are genetic damage events with highest cytotoxicity (10). DSBs occur in human cells ~10 times per day and cell, which impressively reflects the enormous efficiency of the DNA damage detection and repair system of eukaryotic cells (11). The phosphorylation of H2AX serves as one of the first signals for a DSB, and is thus an essential prerequisite for the activation of DNA repair systems, and thus contributes significantly to the stabilization of the genome against genotoxic noxae.

A single DSB leads to the binding of several hundred to thousand γH2A.X proteins to the affected DNA region.

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γH2A.X-DNA binding extends over a length of 0.5-1.7 megabase pairs, so that the entire environment of the DSB is epigenetically labelled (12). These mechanisms are well studied in radiation-induced DSB. Phosphorylation of H2A.X to γH2A.X occurs within minutes after the damage event and is proportional to the dose of ionizing radiation (biodosimetry). Per gray of radiation, ~1% of the H2A.X protein present in the nucleus is phosphorylated (4).

The phosphorylation of H2A.X is not limited to the effect of ionizing radiation (9,13) (Fig. 1). A number of other exogenous noxae can lead to the formation of γH2A.X. Physical noxae include ultraviolet radiation, low pH, and heat stress (14-17). Chemical factors are DNA-damaging agents, such as bleomycin, doxorubicin and reactive oxygen species (18-20). There are also cellular events that can induce H2A.X phosphorylation. During somatic recombination to ensure antibody variability in B cells and for genome stabilization under replicative stress, phosphorylation of H2A.X occurs (15,21,22). Furthermore, DNA damage, including γH2A.X signals also occurs during aging and apoptosis (23-25). In mammalian germ cells, sex chromosomes X and Y are epigenetically silenced by condensing the chromatin of both chromosomes (XY body). In the regulation of this process, gH2A.X is involved along with a number of other factors of DNA repair (26). Since the phosphorylation of the histone variant sometimes occurs independently of DSBs, the characterization of kinetics, number, size and morphology of detected γH2A.X foci is of great relevance (27). The immunocytochemical γH2A.X detection using a phosphor-specific antibody for the phosphorylation on Ser_{139} at the C-terminus of H2A.X is based on the assumption that the intensity of immunofluorescence correlates stoichiometrically with the frequency of DSB (14,27,28). The phosphorylated histone isoform can be detected in so-called γH2A.X foci.

gH2A.X activity is critical for maintaining genome stability in a wide variety of cellular processes. As with all regulatory processes, however, it must also be possible to switch off the corresponding signals. In the case of H2A.X activity, this is dictated by a phosphorylation-dephosphorylation cycle. Phosphorylation is catalyzed by the kinases ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related protein (ATR), DNA-dependent protein kinase (DNA-PK) and mitogen-activated protein (MAP) kinases p38, which can be reversed by the protein phosphatases (PP) PP2A, PP4, PP6 and wild-type p53-induced phosphatase 1 (WIP1) (29-32) (Fig. 2).

3. Phosphorylation of H2A.X in the DNA damage response

The cellular response to detected DNA damage includes both temporary cellular responses, such as checkpoint control activation, as well as permanent cellular responses such as cell cycle arrest and apoptosis (7).

DNA damage detection and repair constitute a complex cellular event involving the entire histone biochemistry. Both the structure and function of nucleosomes are regulated by post-transcriptional modifications to histone proteins. These include acetylation, methylation, ubiquitination and phosphorylation, which orchestrate the signaling pathways involved in DNA repair and induce structural changes in the DNA histone architecture required for this process (33). A key post-translational modification in the context of DNA damage is the phosphorylation of H2A.X by the kinases, ATM, ATR and DNA-PK (9,15,34-36). As serine/threonine kinases, these enzymes belong to the phosphoinositide 3-kinase (PI3K) family and act as sensors for DNA damage (37). PI3K phosphorylate their substrates on the amino acid motif serine or threonine, glutamine, and glutamic acid (Ser/Thr-Gln-Glu) (38).

During DNA damage detection, γH2A.X and DNA repair proteins, such as p53 binding protein 1 (53BP1) and breast cancer 1 (BRCA1) colocate (39,40). The activation of PI3K is still a general reaction to different DNA damage, and only the activation of H2AX by phosphorylation leads to the mobilization of DSB specific signal and repair proteins (41). However, current investigations indicate that both DSB recognition and the recruitment of repair factors are still feasible without γH2A.X (37).

4. γH2A.X as a protein biomarker

Based on the proportionality of γH2A.X formation and radiation dose as utilized in biodosimetry in radiobiology (4), γH2A.X was previously tested as a direct correlate for physically and chemically induced DSB (6). Furthermore, γH2A.X was also tested as a biomarker for incidences correlating with DSB. It has been used as a biomarker of cell death in the presence of chemotherapeutic agents and to detect the genotoxic effect of tobacco smoke in lung cells (18,42).

In the evaluation of the γH2A.X focus assay with primary mouse embryonic fibroblast cells, the test method was found to be as specific and sensitive as the two genotoxic standard procedures, the micronucleus assay and the comet assay (43). H2A.X phosphorylation has been defined as a genotoxic endpoint along with micronucleus formation and mutation frequency. γH2A.X detection is thus also used for clinical monitoring of DNA damage during chemotherapy (44) and for determining patient radiosensitivity (13,45,46).

In oncology, the quantification of γH2A.X can be used to detect precancerous lesions (47). The level of phosphorylated H2AX correlates with cancer-associated genomic instability of tumor cells and could potentially be used as a biomarker for prediction and recurrence (7,47,48). In addition, the DSB-dependent accumulation of γH2A.X can be used as a biodosimeter to determine age (24). In recent years, it has become increasingly clear that H2AX phosphorylation not only serves to detect DNA damage, but also performs an essential task in the processes of chromatin remodeling and thus DNA repair itself. Therefore, in fractionated radiotherapy, there are approaches to individually determine the time intervals by monitoring the gH2A.X status, so that healthy tissue can recover as completely as possible (Table I) (49,50).

5. Conclusion and future perspectives

As discussed above, a growing body of literature demonstrates the importance of H2A.X in DNA damage recognition and repair, but also in genome remodeling processes in general. An essential component of this functionality is the activation and inactivation of the histone by phosphorylation and dephosphorylation. The H2A.X phosphorylation status thus represents

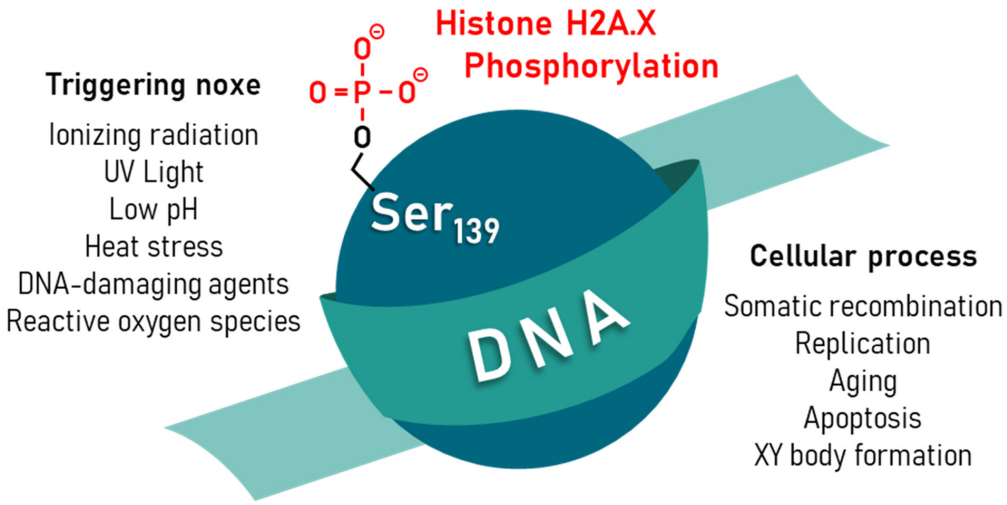

Figure 1. Physical and (bio)chemical stress noxae, such as ionizing radiation, UV light, low pH, heat stress, DNA-damaging agents, and reactive oxygen species can lead to the phosphorylation of serine 139 (Ser_{139}) of histone H2A.X in eukaryotic cells. In addition, physiological cellular processes such as somatic recombination, replication, aging, apoptosis, and XY body formation may also result in Ser_{139}-phosphorylated H2A.X. UV, ultraviolet.

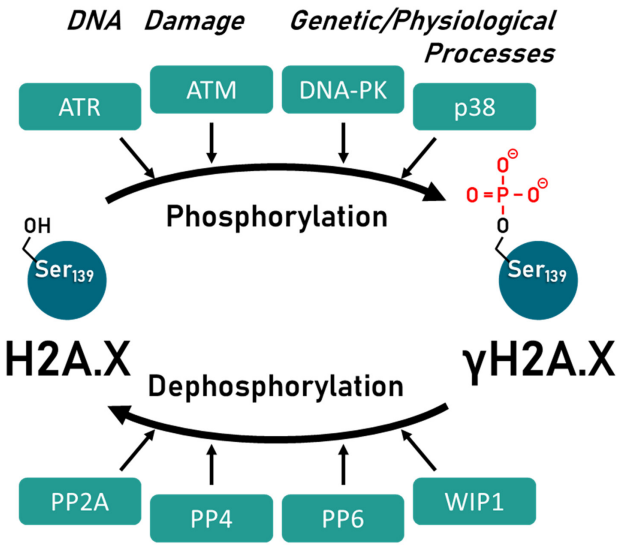

Figure 2. Regulation of H2A.X activity by the phosphorylation-dephosphorylation cycle of various kinases and protein phosphatases. Kinases involved are: ATM, ATR, DNA, MAPK p38. Protein phosphatases involved are: PP2A, PP4, PP6, WIP1. The figure has been modified from a previous study (32). ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; DNA-PK, DNA-dependent protein kinase; MAPK p38, mitogen-activated protein kinase p38; P2A, protein phosphatase 2A; PP4, protein phosphatase 4; PP6, protein phosphatase 6; WIP1, wild-type p53-induced phosphatase 1.

a central sum parameter for the genome integrity of eukaryotic cells and allows conclusions to be drawn about DNA-associated processes in cells and tissues. This can be of great use for applications in life sciences and medicine. As a biomarker for DNA damage and genotoxicity as well as a clinical marker for radiotherapy outcome, drug efficacy, and tissue regeneration, the H2A.X/γH2A.X status is already used or such applications are emerging. Particularly in the field of biological dosimetry, microscopic routine systems for the detection of γH2A.X are already in use, which allow automated, rapid and cost-effective analysis even of large numbers of samples. The establishment of γH2A.X routine analysis in corresponding specialized treatment centers would therefore be easily feasible.

Table I. Current applications of γH2A.X detection in biomedicine.

γH2A.X application	(Refs.)
Approved biological dosimetry	(4,6)
Approved genotoxicity assay	(42,44)
Cytostatics efficacy assay	(18)
Radiotherapy outcome monitoring	(13,45,46)
Detection of precancerous lesions	(47)
Detection of cancer progression	(7,48)
Tissue regeneration monitoring	(49,50)

For further details on these applications, please see main text.

As with all biomarkers, the specificity of γH2A.X detection is of primary importance. The complex cellular mechanisms in which γH2A.X is involved listed in this review article therefore also highlight potential limitations of practical/clinical use. Countering these is therefore an important goal of future γH2A.X research. For example, the combined detection of γH2A.X and 53BP1 significantly increased the precision of DSB-based biodosimetry following radiation exposure. Combining multiple markers thus represents a promising strategy with which to increase the reliability of biomarkers and thereby bring them into practical application.

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Author's contributions

MBS conceptualized, prepared and drafted the manuscript. MBS confirms the authenticity of all the raw data.

Ethics approval and consent to participate

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Patient consent for publication

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Competing interests

The author declares that there are no competing interests.

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