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Review

H2AX: A key player in DNA damage response and a promising target for cancer therapy

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ABSTRACT

Cancer is caused by a complex interaction of factors that interrupt the normal growth and division of cells. At the center of this process is the intricate relationship between DNA damage and the cellular mechanisms responsible for maintaining genomic stability. When DNA damage is not repaired, it can cause genetic mutations that contribute to the initiation and progression of cancer. On the other hand, the DNA damage response system, which involves the phosphorylation of the histone variant H2AX (γH2AX), is crucial in preserving genomic integrity by signaling and facilitating the repair of DNA double-strand breaks. This review provides an explanation of the molecular dynamics of H2AX in the context of DNA damage response. It emphasizes the crucial role of H2AX in recruiting and localizing repair machinery at sites of chromatin damage. The review explains how H2AX phosphorylation, facilitated by the master kinases ATM and ATR, acts as a signal for DNA damage, triggering downstream pathways that govern cell cycle checkpoints, apoptosis, and the cellular fate decision between repair and cell death. The phosphorylation of H2AX is a critical regulatory point, ensuring cell survival by promoting repair or steering cells towards apoptosis in cases of catastrophic genomic damage. Moreover, we explore the therapeutic potential of targeting H2AX in cancer treatment, leveraging its dual function as a biomarker of DNA integrity and a therapeutic target. By delineating the pathways that lead to H2AX phosphorylation and its roles in apoptosis and cell cycle control, we highlight the significance of H2AX as both a prognostic tool and a focal point for therapeutic intervention, offering insights into its utility in enhancing the efficacy of cancer treatments.

1. Introduction

Cancer is a complex and potentially deadly group of diseases characterized by the uncontrolled growth and proliferation of cells that evade the body's regulatory mechanism [1]. The disease originates from a series of genetic and epigenetic changes that disrupt the normal functioning of cells, leading to abnormal cellular behavior [1,2]. Critical to cancer development are mutations in key genes that control cellular growth, such as oncogenes and tumor suppressor genes [1]. These genes normally regulate cell growth, division, and death, allowing tissue integrity and facilitating repair and regeneration [1]. However, various factors, including genetics, environmental exposures, lifestyle choices, diet, infectious agents, and exposure to carcinogens, can disrupt this balance, leading to DNA damage and mutations that increase the risk of cancer [1,3].

H2AX is critical in the complex network of cellular mechanisms and molecular interactions. It is a variant of the histone H2A and a product of the H2AFX gene, emerges as a critical player [4,5]. Representing a variable but significant proportion of the mammalian histone H2A, H2AX is instrumental in nucleosome assembly, chromatin remodeling, and, most pivotally, DNA repair. The phosphorylation of H2AX at the Ser-139 residue produces γH2AX, a critical event in the cellular response

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to DNA double-strand breaks [6,7]. This modification signals the presence of the lesion and activates DNA repair machinery to fix the damage [8–10].

The protein H2AX plays a critical role in ensuring the stability of our genetic material, a function that has been spotlighted due to its connection with cancer development. Specifically, in the context of colorectal cancer, the amount of phosphorylated H2AX within the cancerous tissues has been associated with more aggressive forms of the disease and lower survival rates among patients. This relationship underscores the potential of H2AX as an important indicator that could help predict how the disease progresses and the overall prognosis for patients. [11,12]. γH2AX is more than just a diagnostic tool. It maintains genomic integrity and facilitates DNA repair, providing essential insights into cellular responses under stress and disease states. In radiation biology and pharmaceutical research, it is instrumental in assessing the biological effects of ionizing radiation and evaluating the genomic consequences of chemotherapy and anti-cancer drugs, respectively.[13]. By providing a window into the intricate web of interactions within the genome, γH2AX offers profound implications for therapeutic innovation and our understanding of cellular resilience and vulnerability.

This review provides an in-depth analysis of the complex roles of H2AX, from its detailed structure to its crucial role in cancer treatment. The review aims to shed light on the significant impact of H2AX phosphorylation on cancer development and its management. By examining the molecular mechanisms of H2AX and its potential as a target for therapy, the review offers new perspectives on diagnosing and treating cancer. Ultimately, this contributes to enhancing patient care and outcomes in battling this disease.

2. Structure of H2AX

Histone H2AX, a derivative of the histone H2A protein, plays a crucial role as a constituent of the nucleosome, the fundamental structural unit of chromatin[14–16]. The nucleosome is comprised of an octamer of histone proteins, consisting of two copies each of H2A, H2B, H3, and H4, as depicted in Fig. 1. Each of the four core histones of the nucleosome can bind covalently to various chemical compounds. Over 60 residues in histone peptides contain more than 100 different

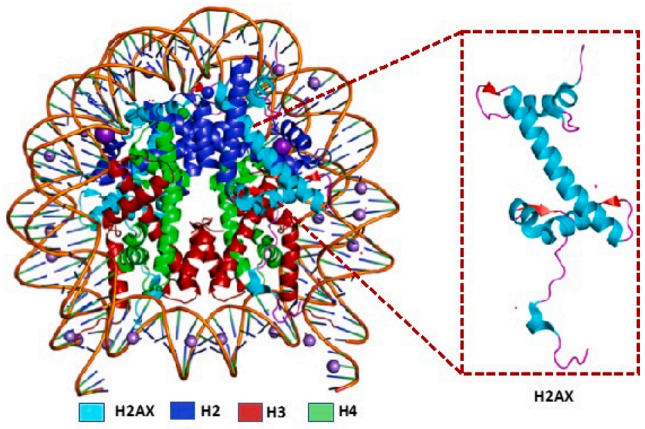

Fig. 1. Detailed view of mammalian core histone modifications. This figure illustrates the structural organization of mammalian core histones: H2A, H2B, H3, and H4, highlighting their globular domains and the projecting N- and C-terminal tails. The nucleosome's octamer structure is depicted, composed of two units each of H2A-H2B and H3-H4, color-coded as light pink, pink, brown, and green, respectively. Surrounding these histones, DNA is elegantly wrapped, demonstrating the foundational structure of chromatin. The figure also details post-translational modifications on the histone tails, including acetylation (blue), methylation (red), phosphorylation (green), and ubiquitination (pink), underscoring the dynamic nature of histone modifications in regulating chromatin architecture and gene expression.

modifications [6]. This structure serves as a foundation for the wrapping of DNA, forming a condensed and well-structured chromatin configuration [17]. The C-terminal tail of H2AX possesses a distinct characteristic: a conserved Ser139 residue, which enables it to undergo phosphorylation upon encountering double-strand breaks (DSBs). This phosphorylation event subsequently results in the synthesis of γH2AX [15–17]. The alteration assumes a critical function in the cellular reaction to DNA damage and is of utmost importance in preserving genomic integrity and facilitating effective DNA repair mechanisms [15,17,18].

Phosphorylating H2AX is triggered in reaction to several forms of DNA damage, such as DSBs, single-strand breaks, and damage generated by ultraviolet (UV) radiation[15]. The γH2AX formation acts as a foundation for the recruitment of DNA damage response (DDR) proteins, such as the Mre11-Rad50-Nbs1 (MRN) complex, 53BP1, and BRCA1, to the specific location of DNA damage[15–17]. These proteins play a crucial role in the restoration of DNA integrity by employing several processes, such as homologous recombination (HR) and non-homologous end joining (NHEJ), to repair DNA damage [15,16]. Besides its involvement in DNA repair, H2AX has also been associated with several cellular functions, such as transcriptional control and chromatin remodeling. [19]. H2AX has been shown to interact with several chromatin remodeling complexes, including the SWI/SNF (SWItch/Sucrose Non-Fermentable) complex and the INO80 (subunit of the chromatin remodeling complex) complex, suggesting a role in the regulation of chromatin structure and gene expression [2]. Furthermore, H2AX has also been documented to play a role in maintaining telomere length and function, which is critical for the stability of the genome [19].

3. H2AX and double-strand DNA breaks

Variations in known genetic pathways cause disruption in the otherwise strictly regulated checkpoint [20,21]. Radiation, chemicals, and biological processes are major causes of DNA damage in eukaryotic cells. H2AX is an essential component of the response of these cells to DSBs. [22,23]. The phosphorylation spreads around the DSB, creating γH2AX foci, a distinctive pattern that signals the recruitment and activation of DNA repair proteins to initiate the repair process [24–26]. H2AX has piqued the interest of scientists due to its presence in extremely sensitive cytogenetic areas, which is known to be frequently altered in most human malignancies, including lymphomas, leukemia, and breast cancer [5,6]. Histone H2AX phosphorylation is a sensitive marker for DSB, which contributes to both genomic instability and cancer treatment; therefore, tracking its generation may be a sensitive way to track cancer progression and treatment effects [9,27]. Research has demonstrated that the subsequent development of γH2AX foci is utilized as reliable markers for the presence of DNA damage [28]. H2AX-specific antibodies enable the visualization of a "focus" at the DSB site [13]. Monitoring the levels of γH2AX foci can provide valuable information about the extent of DNA damage and the efficiency of repair processes [29]. The presence of H2AX in chromatin can be observed quickly after DSB induction as distinct nuclear foci. The frequency of foci indicates DSB as each focus represents a single DSB [30]. Microscopy, flow cytometry, and Western blotting of cell/tissue lysates can be used to detect H2AX-containing nuclear foci, with overall H2AX levels normalized. H2AX phosphorylation can be determined in individual cells with high sensitivity and accuracy using multiparameter flow or laser scanning cytometry, and H2AX expression in cell populations can be correlated with DNA content or induction of apoptosis [2,30,31].

4. Association of H2AX and ATM

The apical kinase known as ATM has a crucial role in coordinating various cellular responses to DSBs, such as DNA repair and activation of checkpoints. The protein under consideration serves as a pivotal regulator of the cellular response to DNA damage, hence playing a critical

role in preserving the integrity of the genome [32]. The activation of ATM triggers the phosphorylation and plays a crucial role in developing γH2AX foci and recruiting repair proteins to the specific location of DNA damage. The phosphorylation of H2AX leads to the activation of the DNA damage checkpoint, whereas its dephosphorylation is essential for reducing the intensity of the checkpoint response [33]. Both ATM and ATR can convey their effects through P53, either directly or via activation of checkpoint kinase 2 (Fig. 2). P53 activates the CDK2 inhibitor P21, which blocks damaged cells from progressing to the S phase of the cell cycle [26]. In cases where ATM (ataxia-telangiectasia mutated) is absent, DNA-PK (DNA-dependent protein kinase) steps in to phosphorylate histone H2AX, transforming it into γ-H2AX [34]. The formation of γ-H2AX foci plays a crucial role in facilitating accurate DNA repair, ensuring the integrity of the genetic material is maintained. Following exposure to ionizing radiation or harmful chemical agents, γH2AX rapidly assembles at chromatin areas surrounding DSBs, showcasing its vital role in the cell's defensive mechanism against DNA damage induced by various genotoxic agents. This protein is a key component of chromatin, playing an instrumental part in DNA repair processes. The repair of DSBs is achieved through at least five distinct mechanisms: break-induced replication, homologous recombination (HR), alternative non-homologous end joining, single-strand annealing, and canonical non-homologous end joining [26].

In this complex repair landscape, γH2AX functions as a signal amplifier, enhancing the DNA damage response and recruiting an array of proteins essential for both non-homologous end joining and homologous recombination to the site of damage, thus facilitating the precise repair of the genome.[33].

5. Activation of H2AX by genotoxic agents

Genotoxic agents that damage DNA can be classified in several different ways: they can be endogenous, exogenous, physical in nature, such as ultraviolet (UV) light and ionizing radiation, or chemical in nature, such as reactive oxygen species (ROS), intercalating agents, Topoisomerase inhibitors, Platinum drugs and base analogs which can cause direct or indirect DNA damage, or both. Furthermore, DNA-damaging chemicals are frequently employed to treat cancer, and understanding how cells respond to them is critical to boost their efficacy [35,36].

Many of the cytotoxic drugs (chemotherapy) widely used to treat cancer patients induce significant DNA damage, which causes cell cycle checkpoints to be activated, resulting in cell death/arrest [37]. Anti-cancer medications such as DNA replication inhibitors, cross-linking agents, and topoisomerase inhibitors can generate DSBs, leading to γH2AX production due to replication and transcriptional stressful events [7,37,38].The various intrastrand cross-links caused by cisplatin have multiple effects on a cell, eventually leading to DNA

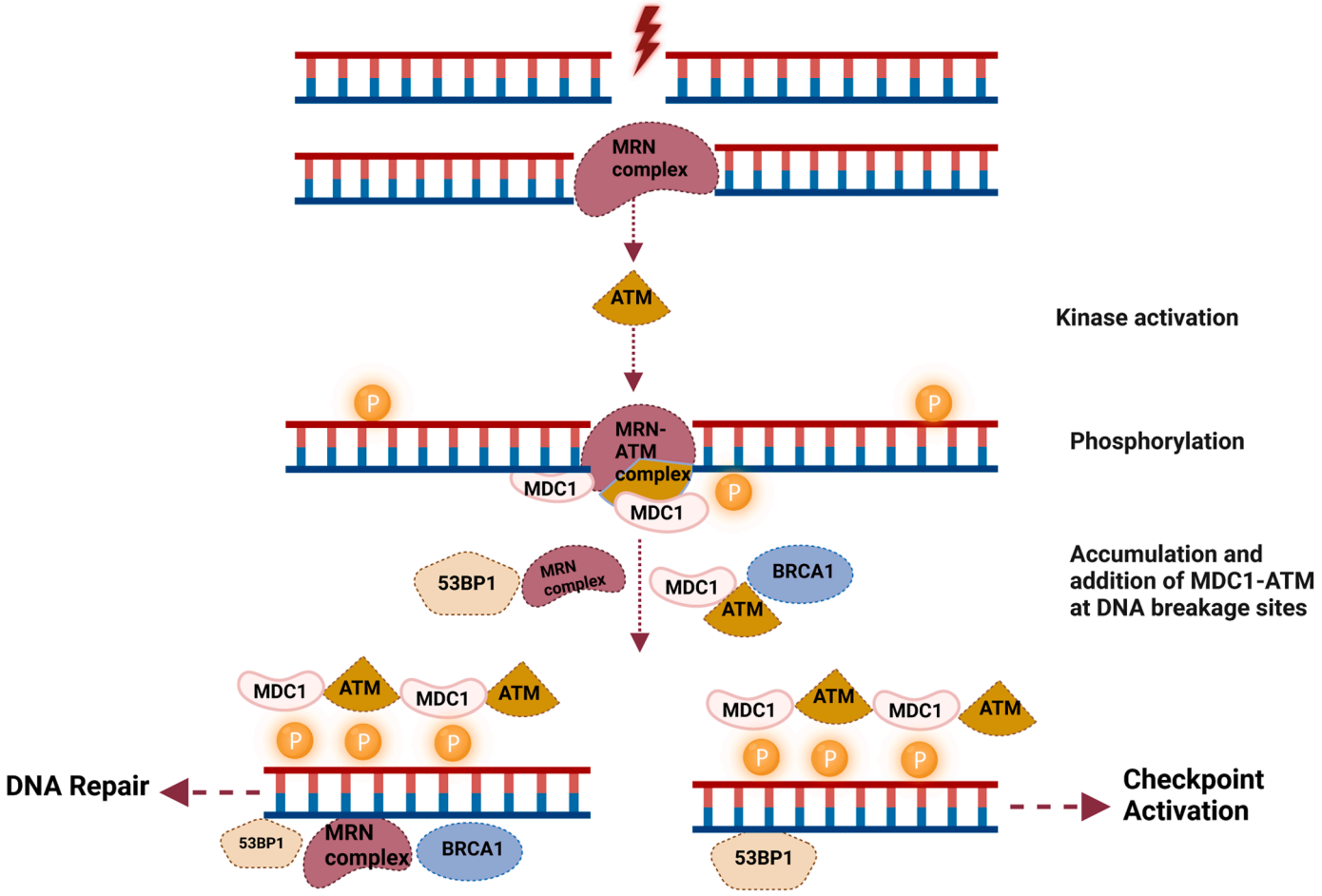

Fig. 2. The DNA damage response pathway. This figure provides a comprehensive overview of the DNA damage response triggered by double-strand breaks. Mutagens can induce these breaks within DNA that is closely associated with histone proteins. Upon DNA damage, the MRN complex, consisting of MRe11, Rad50, and Nbs1 proteins, is recruited to the site, initiating the repair process. This complex then activates ATM kinase, which in turn phosphorylates the H2AX histone protein, generating γ-H2AX. The phosphorylated H2AX facilitates the recruitment and accumulation of MDC1 and ATM at the damage sites, which are crucial steps for the subsequent recruitment of other repair proteins like BRCA1 and 53BP1, thereby orchestrating the DNA repair mechanisms and cell cycle checkpoints to maintain genomic integrity.

strand breaks and Gamma H2AX formation[38]. Topoisomerase II inhibitors directly induce DSBs, whereas Topoisomerase I inhibitors first generate SSBs (single-strand breaks), which are transformed to DSBs when they encounter a replication fork [39–42].

The γH2AX assay is a valuable method for evaluating the genotoxicity of different agents, encompassing both chemical and physical stressors [13]. Previous studies have demonstrated that the γH2AX assay can detect DNA damage caused by both genotoxic and nongenotoxic substances [43]. The assay relies on identifying γH2AX foci, which are generated at the location of DSBs. The quantity and severity of γH2AX foci directly correlate with the magnitude of DNA damage [13,43].

6. Oxidative stress and H2AX protein

In the context of oxidative stress, the presence of reactive oxygen species (ROS) has the potential to cause DNA damage, resulting in the formation of DNA lesions such as double-strand breaks. This, in turn, triggers the phosphorylation of H2AX, leading to the formation of γH2AX [44]. The interplay between oxidative stress and H2AX highlights the importance of H2AX as a key player in cellular DNA damage response mechanisms [45]. Understanding this relationship may provide insights into various biological processes, including aging, cancer development, and other diseases associated with oxidative stress-induced DNA damage. Consequently, investigating strategies to modulate H2AX function under oxidative stress conditions could hold therapeutic potential for addressing conditions linked to DNA damage and impaired repair mechanisms [45–47].

Research has shown that oxidative stress can induce H2AX phosphorylation in human spermatozoa through DSB induction and that γH2AX may be used as a sensitive, novel marker for such double-strand breaks [48,49]. Additionally, chronic oxidative stress can promote H2AX protein degradation and enhance chemosensitivity in breast cancer patients [46]. Investigating methods to regulate H2AX activity during oxidative stress may offer potential therapeutic advantages in addressing conditions linked to DNA damage and impaired repair mechanisms. In addition, it can enhance the effectiveness of chemotherapy in cancer patients [46,47,50].

7. Association of H2AX and Apoptosis

H2AX, a variant of the histone H2A, is critically acclaimed for its role in the cellular response to DNA damage. Upon DNA strand breaks, H2AX is rapidly phosphorylated at the site of damage, a modification that facilitates the recruitment of DNA repair proteins, thereby promoting cell survival [2,51,52]. This initial response underscores the fundamental role of H2AX in maintaining genomic stability.

The functionality of H2AX extends significantly beyond its role in DNA repair, as evidenced by its intricate involvement in programmed cell death or apoptosis. Apoptosis is a vital cellular mechanism that eradicates cells which are damaged or no longer necessary, doing so in a manner that avoids the release of potentially inflammatory intracellular contents [53] [54]. The activation patterns of H2AX during apoptosis are unique and signify a complex regulatory role that transcends its DNA repair functions.

Emerging research has highlighted H2AX's pivotal role in apoptosis regulation. H2AX undergoes specific post-translational modifications in response to apoptosis-inducing stimuli, which are essential for the initiation and execution of programmed cell death [55,56]. These modifications signal the cell to transition from a repair-focused response to the activation of apoptotic pathways, thereby maintaining a critical balance between survival and death. The exact mechanisms through which H2AX influences apoptosis involve intricate interactions with other proteins and signaling pathways, which are still under active investigation [57].

Research has unraveled that H2AX's activation during apoptosis is not coincidental but a highly orchestrated event. The modulation of H2AX in response to apoptotic signals is influenced by various factors, including the nature and intensity of cellular stressors like radiation, oxidative stress, and viral infections. These factors induce distinct patterns of H2AX phosphorylation, reflecting its sensitivity and adaptability to different types of cellular insults [53]. Moreover, the interplay between DNA repair and apoptosis reveals that H2AX serves as a molecular crossroad: steering the cell towards apoptosis in cases of severe DNA damage, thereby preventing the propagation of harmful mutations, or towards repair and survival when damage is minimal and recoverable. (Fig. 3). The elucidation of H2AX's involvement in apoptosis not only advances our understanding of cellular decision-making processes but also opens new therapeutic horizons, particularly in cancer, where apoptosis dysregulation is a hallmark [58,59]. Despite these advances, numerous questions remain, particularly regarding the specific signaling pathways and molecular interactions through which H2AX modulates apoptosis. Understanding the crosstalk between H2AX and other regulators of apoptosis is crucial for a comprehensive grasp of its role in cell fate decisions, paving the way for innovative therapeutic interventions in diseases characterized by apoptosis impairment. While significant strides have been made in elucidating the role of H2AX in apoptosis, several aspects remain to be explored. The exact molecular interactions and signaling pathways through which H2AX mediates its effects on apoptosis necessitate further investigation. Moreover, understanding the crosstalk between H2AX and other apoptotic regulators will be crucial in fully deciphering the complex regulatory network governing cellular responses to DNA damage and stress.

8. Mechanistic Interaction between H2AX and DSBs

The interaction between H2AX and DSBs is a cornerstone of cellular DNA damage response mechanisms, pivotal for maintaining genomic stability. When DSBs occur, a rapid and highly coordinated series of events is triggered, with H2AX playing a central role. Upon the induction of DSBs, the ATM kinase is rapidly activated and recruited to the site of breaks. ATR and DNA-PK can also contribute to H2AX phosphorylation under certain contexts [60,61]. The phosphorylation occurs primarily at the serine 139 residue of H2AX, creating γH2AX [62]. This modification serves as a beacon, marking the vicinity of DSBs and facilitating the recruitment of DNA repair machinery.The phosphorylation of H2AX leads to the nucleation of γH2AX foci, which expand along the chromatin surrounding the DSBs. These foci can extend over large chromatin domains, amplifying the signal from the damage site and providing a scaffold for the assembly of repair complexes [63]. The γH2AX foci facilitate the sequential binding and recruitment of multiple DNA repair factors. The MDC1 protein, which directly binds to γH2AX, acts as a platform for further recruitment of repair proteins. This includes the MRN complex, which is involved in DSB end resection, and mediator proteins like 53BP1 and BRCA1, which influence the choice of repair pathway [64,65]. The recruitment of the MRN complex and ATM to γH2AX sites leads to further phosphorylation events, amplifying the DNA damage signal. This cascade ensures the propagation of the damage signal and the maintenance of a robust response to DSBs [66]. γH2AX is pivotal in both homologous recombination (HR) and non-homologous end joining (NHEJ) pathways. In HR, γH2AX facilitates the recruitment of RAD51, promoting strand invasion and homologous pairing. In NHEJ, γH2AX supports the protection of DNA ends and the recruitment of ligase complexes necessary for end ligation [23,67]. Following repair, γH2AX foci are resolved through the action of phosphatases, including PP2A and WIP1, which dephosphorylate γH2AX, signaling the completion of repair and the restoration of genomic integrity [67].

9. Effect of Natural and synthetic drugs on PH2AX

Previous studies have reported that various anti-cancer drugs of both synthetic and natural origin acting through many different pathways directly or indirectly affect PH2AX (Table 1). *Wang H et al.* (2005) found

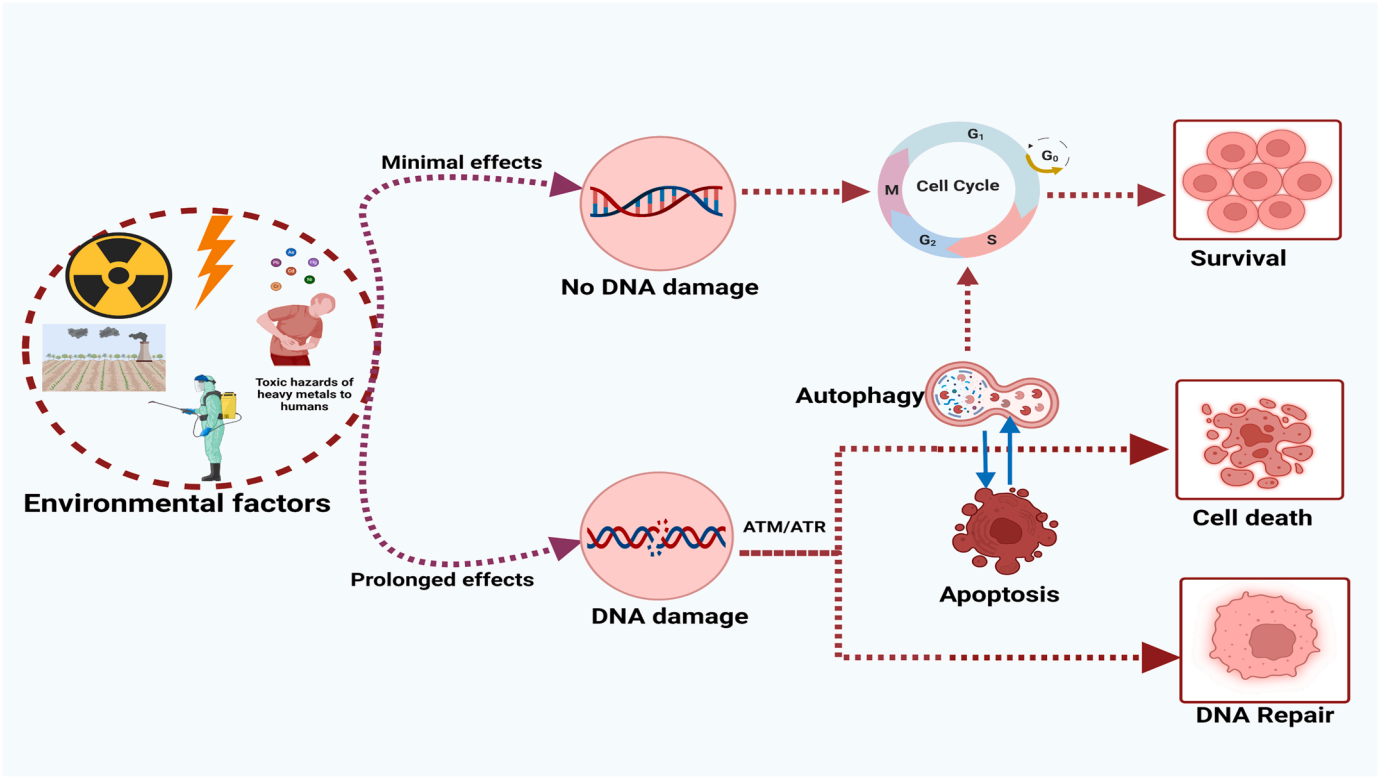

Fig. 3. Comprehensive analysis of environmental impact on DNA damage and repair processes. This detailed figure delineates the multifaceted effects of environmental factors on cellular DNA under conditions of short-term and long-term exposure. It graphically illustrates how varying environmental stimuli can initiate a spectrum of cellular responses, impacting the intricate machinery of DNA damage and repair. In the context of short-term exposure, the figure showcases the immediate cellular mechanisms activated to counteract DNA damage, highlighting the efficient mobilization of DNA repair factors and the activation of cell cycle checkpoints that work in concert to maintain genomic stability. Conversely, the long-term exposure underscores the chronic stress exerted on cellular systems, leading to persistent DNA damage that may overwhelm the repair capabilities, resulting in cell death or triggering survival pathways such as autophagy. Additionally, the figure elaborates on how different environmental stimuli can activate various signaling pathways, influencing the cell's fate through mechanisms like apoptosis, autophagy, or the sustained activation of DNA repair processes and cell cycle checkpoints. This visual representation emphasizes the dynamic interplay between environmental factors and cellular responses, offering insights into the complexity of cellular mechanisms governing DNA integrity and cell viability under diverse exposure scenarios.

that when inhibitors such as wortmannin when used at dosages that are expected to inhibit both ATM and DNA-PK, H2AX phosphorylation is delayed [68]. Studies have stated that Histone deacetylase inhibitors (HDACI) like Vorinostat, etc., have not only been traditionally postulated to target many different non-histone HDAC substrates involved in the regulation of many physiological activities, including cell cycle, cell proliferation, and cell death but also to restore the balance between histone acetylation and regulation of gene expression. Another study discovered that combining HDACIs and Resveratrol leads to increased DNA damage, mitochondrial injury, and the activation of Caspase-3, Caspase-9, and Caspase-8. Moreover, in contrast to the limited induction of H2AX by resveratrol or HDACIs (LBH-589 or vorinostat) when given alone for around 16 h, H2AX expression was significantly elevated in acute myelogenous leukemia cells after receiving combined therapies. [69,70]. Cancer treatment has traditionally made extensive use of natural products and their derivatives as well. Cantharidin, a powerful and specific protein phosphatase 2 A (PP2A) inhibitor, is the active ingredient in Mylabris and is crucial for controlling the cell cycle, apoptosis, proliferation, and determination of cell fate [71]. A study found that through numerous pathways downstream of PP2A, cantharidin reduced gene expression associated with DNA damage repair [72]. According to a second study, cantharidin could increase the cytotoxicity of gemcitabine and erlotinib against pancreatic cancer cells. They monitored the dynamic production of PH2AX, which is produced when cantharidin is combined with radiation to trigger DNA damage [67]. Therefore, cantharidin alone did not cause DNA damage, but cantharidin co-treatment intensified the phosphorylation of histone H2AX, increasing the frequency of PH2AX foci [67,72]. Table 1 discusses various anti-cancer drugs and the mechanism through which they trigger PH2AX. Many are still in ongoing clinical trials, while some are assessed as novel drugs and are being used alone or in combinations with other established therapies.

10. Advancements and obstacles in Utilizing H2AX for cancer treatment

Recent therapeutic strategies have spotlighted the histone variant H2AX as a crucial player in the DNA damage response (DDR) and apoptosis pathways, positioning it as a significant target in cancer treatment. The role of H2AX in cancer progression has been increasingly recognized, with studies linking its enhanced expression to a myriad of cancer-promoting processes. For instance, increased H2AX expression correlates with a heightened DNA repair capacity, activation of MYC signaling, and an amplified ability for cell proliferation and metastasis. Notably, research in breast cancer cells demonstrated that H2AX knockdown resulted in decreased cell proliferation and higher H2AX mRNA levels were observed in metastatic clones and tissues, underscoring its potential as a target in cancer therapeutics[73].

In colorectal cancer (CRC), the elevated mRNA levels of H2AX and the increased presence of γ-H2AX in cancer tissues relative to normal tissues signify a possible universal association of γ-H2AX with cancer progression[9,74]. This association is further emphasized by the link

Table 1
Anticancer agents (Synthetic and Natural) and their mechanism of action via H2AX activation.

Synthetic Drug	Class of drugs	Mechanism of action	References
Olaparib	Poly (ADP-ribose) polymerase 1 (PARP1) inhibitor	Initiates DNA repair by inhibiting PARP	[69]
Rapamycin in Combination with Olaparib	mTOR inhibitor delays entry into senescence in cells treated with chemical stress	Inhibits DNA damage by mitigating p16 and p21	[70]
Doxorubicin Combination with Olaparib	Anthracycline antibiotic	Inhibits topoisomerase II	[69]
Romidepsin (FK228)	Histone deacetylase inhibitors (HDACi)	Targets chromatin-modifying enzymes related to DDR	[71]
Cisplatin	Alkylating agent and IR	Repair by NHEJ and crosslinks the DNA	[71]
Imatinib	Tyrosine kinase inhibitors	Activates caspase-3 and induces apoptosis	[72]
Halaven - pimozide HAL-PIM	Anti-psychotic Drug Mitotic inhibitors	Arrest cell cycle at G2 phase arrest and increases apoptosis	[73]
Panobinostat (LBH589)	HDACi	Inhibits Histone Deacetylase 1	[74]
Mitoxantrone	Topoisomerase II (topo2) inhibitor	Inhibits topoisomerase II and triggers apoptosis	[75]
Clofarabine and cyclophosphamide	Deoxyadenosine analog and alkylating agent	Ribonucleotide reductase inhibition and apoptosis induction	[76]
Gemcitabine and hydroxyurea	Deoxynucleoside analogue and antimetabolites	Trigger phosphorylation of H2AX	[77]
SJG-136	Pyrrolobenzodiazepine dimer and DNA cross-linking agent	Cross-links DNA, activating DDR	[78]
Combination of veliparib (ABT-888) with topotecan	PARP inhibitor and topoisomerase II (topo2) inhibitor	PARP and topoisomerase I–targeted inhibition	[79]
Combination of tipifarnib and etoposide	Nonpeptidomimetic methylquinolinone Farnesyltransferase inhibitors (FTIs)	Catalyzes the transfer of farnesyl moiety inhibiting farnesylation of these polypeptides	[80]
Combination of 5-azacytidine and entinostat	DNMT inhibitor and HDAC inhibitor	Inhibits methyltransferase, resulting in cell death	[81]
Neocarzinostatin	Chromoprotein antitumor antibiotics	Mediates strand breakage of DNA	[43]
Bleomycin	Glycopeptide antibiotic	Inhibition of DNA synthesis	[43]
Tirapazamine	N-oxide HAP and benzotriazine-di-N-oxide class of hypoxic cytotoxins	Generation of free radical species to produce DNA breaks	[43]
Benzo[*a*]pyrene (BaP)	Polycyclic Aromatic Hydrocarbons (PAHs)	Induce γH2AX, ATM, ATM- and Rad3-related (ATR) and DNA-PK in response to DNA damage	[82]
AK301	Kinase inhibitors and piperazine-based mitotic inhibitors	Mitosis-associated DDR, including ATM activation, γH2AX phosphorylation	[60]
MHY407, doxorubicin-etoposide	Carbazole derivative	Increases p21 and cell cycle arrest at the S phase	[83]
Vorinostat	HDACi	Target chromatin-modifying enzymes associated with DNA damage and repair	[84]
Natural Compound	**Origin**	**Mechanism of Action**	**References**
Xanthatin	Sesquiterpene lactone purified from Xanthium strumarium L	Suppress DNA replication & increase the number of H2AX nuclear foci	[85]
Bleomycin	Produced by Streptomyces verticillis	Less inhibition of RNA and protein synthesis	[86]
Resveratrol	Polyphenolic chemical in grapes, berries, peanuts, and other plant sources, red wine	Reduces the capacity of topoisomerase II	[87,88]
Curcumin	Rhizome of turmeric	Increases the proteins of the base excision repair and NHEJ	[89,90]
Epigallocatechin gallate	Polyphenolic component of green tea extracts	Inhibits DNA methyltransferase activity and regulates histone acetylation.	[91]
Triptolide	Chinese medicinal plant thunder god vine	Inhibits the ATPase activity of XPB helicase to affect the nucleotide excision repair pathway.	[92]
Quercetin	Flavonoid present fruits and vegetables.	Block ATM activation and increase antioxidant response by modulating NRF2 signaling	[93]
Berberine	Isoquinoline alkaloid from the Chinese herb Coptis chinensis	Induce oxidative DNA damage, alter RAD51 expression.	[89]
Genistein	Isoflavonoid from soy-based foods	Inhibits DNA-PKcs phosphorylation, repress NHEJ and delay HR repair processes.	[94]
Thymoquinone	Nigella sativa Linn seed extracts	Telomere shortening caused by a DNA-PKcs dependent process.	[89]
Honokiol	Biphenolic compound from the Magnolia officinalis plant	Inhibit the activity of X-family polymerases, involved in the base excision repair process.	[89,95]
Ellagic acid	Fruits and vegetables	Reduce endogenous oxidative DNA damage	[96]
Celastrol	Polyphenolic compound isolated from plants in the Celastraceae family	Induces proteasomal degradation of FANCD2	[97]
Cantharidin	Terpenoid class secreted by blister beetles	Increase levels of phosphorylated H2AX	[64]
Garcinol	Polyisoprenylated benzophenone derivative of the fruit rind Garcinia indica	Blocks chromatin remodelling, can inhibit the NHEJ mechanism	[89,98]
B carotene	yellow, orange, and green leafy fruits and vegetables like carrots, spinach	Reduces level of Ku proteins, implicated in DSB breaks	[99]
3,3'-diindolylmethane	Indole-3-carbinol and found in cruciferous vegetables	AR target genes involved in DNA repair	[100]
Kaempferol	Flavonoid found in vegetables and fruits such as berries, grapefruit	Lowers the expression of ATM and ATR	[100]
Luteolin	Flavonoid enriched in various vegetables and plants such as carrots, broccoli, and parsley	Activates DDR by phosphorylating ATM and H2AX	[101]
Withanolide D	Withania somnifera	Inhibit DNA damage by NHEJ repair pathway	[102]
Isoorientin	Flavonoid extracted from many plant species, such as flax straw, watery leaf	Induce DSBs and supress HR repair	[103]
Harmine	Peganum harmala seeds	Suppress HR repair	[104]
Ferulic acid	Phenolic compound found in many plants	Reduces development of RAD51 foci	[89]
Capsaicin	Chili peppers of Capsicum genus	AKT inactivation and ERCC1 downregulation	[105]
β-Thujaplicin	Monoterpenoid found in the wood of trees of Cupressaceae family	Prevents the development of RAD51 foci and maintains RPA phosphorylation.	[89]
Retiegeric acid	Lichen	Affect nucleotide excision repair and mismatch repair	[89]

between H2AX expression and microsatellite instability (MSI), a known mechanism in carcinogenesis due to mismatch repair deficiency. Such insights into H2AX's role in CRC offer a glimpse into its broader implications in cancer biology and treatment, particularly in the context of immunotherapy efficacy[74–77].

Integrating these findings into the current landscape of cancer treatment, H2AX emerges as a pivotal target in the design of new therapeutic strategies. These strategies encompass the development of drugs impacting H2AX function, which are being evaluated across various stages, from clinical trials to early-stage research. The potential of synthetic lethality, particularly in combination with chemotherapy or radiotherapy, is being explored with the aim of targeting key enzymes in the DDR pathway, such as ATM, ATR, DNA-PK, and PARP. [9,78].

However, the targeting of DDR pathway regulators, primarily scaffold proteins essential for signal transduction, presents a significant challenge. The absence of enzymatic activity in these proteins complicates the creation of small-molecule inhibitors. This is where Proteolysis Targeting Chimeras (PROTACs) come into play. PROTACs, a novel class of molecules, leverage the cell's ubiquitination machinery to selectively degrade target proteins, including those within the DDR pathway. The efficacy of PROTACs in targeting crucial proteins like CMYC and BET illustrates the potential of this approach in advancing cancer therapy, offering a promising avenue to overcome the obstacles of targeting scaffold proteins within the DDR pathway.[79,80]. The integration of H2AX-focused research into therapeutic development not only aligns with the dynamic nature of oncological research but also exemplifies the ongoing endeavor to enhance cancer treatment efficacy[78]. By addressing the challenges associated with targeting the DDR pathway's non-enzymatic components and leveraging the insights gained from H2AX's role in cancer, the field is poised to unlock innovative treatment avenues, potentially transforming patient outcomes in the realm of oncology.

11. Conclusion and future perspective

H2AX is a fundamental histone variant that plays a crucial role in advancing research in molecular biology and genetics. It has proven to be an important factor in maintaining genome integrity and responding to DNA damage, which has significant therapeutic implications. While its role in DNA damage response is well-established, new studies continue to reveal its potential beyond these basic functions. H2AX's involvement in cellular defense mechanisms, especially through its phosphorylation at sites of DNA DSBs, highlights its vital role in maintaining cellular viability and genomic stability.

The process of H2AX phosphorylation, which marks the beginning of DNA repair pathways, is currently a major research focus. This process indicates that cells are attempting to repair genomic damage and acts as a signal to recruit the necessary repair machinery. By understanding the intricacies of this signaling cascade, researchers hope to identify new targets for therapeutic intervention. This will provide a blueprint for developing strategies to suppress tumor growth by exploiting DDR mechanism pathways.

Furthermore, the function of H2AX in protecting the stability of the genome is crucial in preventing cancerous transformation. The protein's ability to effectively repair damaged DNA and prevent the buildup of harmful lesions demonstrates its potential as a target for cancer prevention and treatment. This protective mechanism emphasizes the importance of H2AX in preventing cancer development and provides a promising area for research into cancer prevention.

Recent studies indicate that H2AX has distinct functions in different cellular contexts, suggesting its versatility beyond the conventional DNA repair mechanisms. These context-specific roles, possibly influenced by DNA DSBs, offer new insights into tissue-specific repair mechanisms. Further research into these unique functions could shed light on the complexities of H2AX's involvement in various biological processes and its impact on tissue homeostasis and disease.

Furthermore, the fact that H2AX is present during embryonic development in various species emphasizes its critical function in embryogenesis. Exploring the intricacies of H2AX activity during this crucial developmental period may uncover fundamental knowledge about cellular differentiation and organogenesis mechanisms, which could lead to significant advancements in regenerative medicine and developmental biology.

The study of H2AX in human cancers is expected to provide important insights into its role in cancer biology beyond DNA damage signaling. By comprehending the various ways H2AX can be dysregulated in different types of cancer, we may discover new diagnostic markers and therapeutic targets, which could lead to more effective treatments for cancer.

Lastly, utilizing the γH2AX assay as a biodosimetry tool exemplifies the translational potential of H2AX research. Enhancing the precision and efficiency of radiation exposure assessment through this assay could significantly impact public health, particularly in emergency response scenarios.

As we gaze into the future, the horizon of opportunities for H2AX research is broad and full of promise. The ongoing exploration of its multifaceted roles, from DNA repair to cancer therapy and beyond, continues to enrich our understanding of cellular biology. The potential for H2AX to inspire novel therapeutic strategies and diagnostic tools is immense, heralding a future where the intricate details of cellular repair mechanisms are harnessed to advance human health and disease management.

Author contributions

KSP and SU designed the manuscript content and wrote the manuscript. SK, NA, UH, ZM, MS & AAB helped write and review the manuscript. All authors read and approved the final manuscript before submission.

Ethical statement

Not Applicable

CRediT authorship contribution statement

Kirti S. Prabhu: Writing – review & editing, Writing – original draft, Conceptualization. **Shilpa Kuttikrishnan:** Writing – review & editing. **Nuha Ahmad:** Writing – review & editing. **Ummu Habeeba:** Writing – review & editing. **Zahwa Mariyam:** Writing – review & editing. **Muhammad Suleman:** Writing – review & editing. **Ajaz A. Bhat:** Writing – review & editing. **Shahab Uddin:** Writing – review & editing, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

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