Review

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gamma-H2AX: Can it be established as a classical cancer prognostic factor?

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Abstract

Double-strand breaks are among the first procedures taking place in cancer formation and progression as a result of endogenic and exogenic factors. The histone variant H2AX undergoes phosphorylation at serine 139 due to double-strand breaks, and the gamma-H2AX is formatted as a result of genomic instability. The detection of gamma-H2AX can potentially serve as a biomarker for transformation of normal tissue to premalignant and consequently to malignant tissues. gamma-H2AX has already been investigated in a variety of cancer types, including breast, lung, colon, cervix, and ovary cancers. The prognostic value of gamma-H2AX is indicated in certain cancer types, such as breast or endometrial cancer, but further investigation is needed to establish gamma-H2AX as a prognostic marker. This review outlines the role of gamma-H2AX in cell cycle, and its formation as a result of DNA damage. We investigate the role of gamma-H2AX formation in several cancer types and its correlation with other prognostic factors, and we try to find out whether it fulfills the requirements for its establishment as a classical cancer prognostic factor.

Keywords

gamma-H2AX, cancer, prognosis, histone, DNA damage

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Introduction

Cancer is a leading cause of morbidity and mortality worldwide. Approximately, 15 million patients present as new cancer cases every year with a male/female ratio of 10:9. It is predicted that there will be 23.6 million new cancer cases worldwide each year by 2030, if recent trends in prevalence of major cancers and population growth are seen globally in the future. Lungs, female breast, bowel, and prostate are the four most common sites of cancer development, accounting for 42% of all new cases.[1] Regarding the aspects previously mentioned, the need of the early and constructed management is crucial. Biomarkers specific to the particular patient and cancer type are cornerstones for the individual management planning, contributing to the early diagnosis and good prognosis.

gamma-H2AX (gH2AX) is the phosphorylated form of histone H2AX and can function as a sensitive marker for double-strand breaks (DSBs), which signifies genomic instability and can potentially contribute to cancer initiation and progression. In this way, monitoring the formation of gH2AX can potentially be a sensitive means of cancer development detection.

In this review, we summarized the available data from retrospective, prospective, and randomized trials on gH2AX to provide clinicians and other health care professionals with a succinct and focused update.

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Materials and methods

The MEDLINE/PubMed, OVID, and Google Scholar databases were searched for publications with both the medical subject "gH2AX" and keywords "cancer" or "histone" or "prognosis" or "DNA damage." The search was conducted on the basis of both the Medical Subject Heading (MeSH) tree and text search. We restricted our search to English language, till August 2016. Case series and reviews of the literature were included in the study. We sought to review all updates on the subject after the introduction of gH2AX in the oncological armamentarium.

Results

gH2AX in cell cycle

H2AX is a variant of histone H2A. H2A histone together with H2B, H3, and H4 makes an octamer of core histone proteins, around which 145–147 base pairs of DNA are wrapped. This unit is called as nucleosome, which is the smallest subunit of genomic DNA in eukaryotic cells.[2]

The H2AX variant represents 2%–25% of total H2A and its levels are cell line and tissue dependent.[3] The fact that the gene encoding H2AX contains landscapes of both replication-dependent and replication-independent histone species makes this gene unique among the other histone genes. Through this mechanism of translational regulation, the presence of sufficient H2AX molecules at G0 and G1 cell cycle is ensured.[4]

DNA damage occurs as a result of a variety of events,[5] such as ionizing irradiation, treatment with radiomimetic agents, drugs, or even as a part of normal physiological processes, with meiosis and class-switch recombination among them. Retroviral infection with subsequent integration or telomere shortening also causes DNA damage. DSBs may occur in any of the cases mentioned above and can cause the most lethal type of DNA damage.[6–8]

DSBs in their turn are responsible for the activation of three phosphatidylinositol 3-kinase-like kinases (PIKKs): ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), and DNA-dependent protein kinase (DNA-PK), which catalyze the H2AX phosphorylation.[9,10] ATM and DNA-PKs display functional redundancy in phosphorylating H2AX following ionizing radiation, while ATR is more important for H2AX phosphorylation in response to DNA damage that would slow or stall replication forks.[4] H2AX is phosphorylated at serine 139 in the C terminus (Figure 1). H2AX, Nijmegen breakage syndrome 1 (nibrin/NBS1), and mediator of DNA damage checkpoint protein 1 (MDC1) create a signal amplification loop which also leads to H2AX phosphorylation upto megabase regions surrounding DSBs.[11] NBS1 and MDC1 bind directly to gH2AX through MDC1 BRCT domain and this long-range gH2AX/MDC1 localization adjacent to DSBs serves as a landing site for the accumulation of other discoidin domain receptor (DDR) proteins, including the MRN (MRE11-RAD50-NBS1) complex, ring finger protein 8, E3 ubiquitin protein ligase (RNF8), breast cancer 1 (BRCA1), early onset (BRCA1), and p53-binding protein 1 (53BP1).[12–16] It has also been suggested that in addition to this functional role of gH2AX as a response to DSBs, gH2AX also plays a critical structural role. More specifically, gH2AX contributes to the timely efficient retention of remodeling factors at the repair site.[17,18] It is also possible that gH2AX and the associated proteins that it helps to accumulate may assist in holding broken ends together, thereby allowing time for DNA repair and minimizing the risk of misrepair.[19,20] Another important DSBs repair pathway includes the recruitment of cohesins (a protein complex that regulates the separation of sister chromatids during cell division, either mitosis or meiosis) to the site of DNA damage to promote sister chromatid–dependent recombinational repair, through maintenance of sister chromatids and by holding the damaged chromatid close to its undamaged sister template after DNA replication. The conservation of the above mechanism would lead to an undamaged sister chromatin mediating an error-free recombination repair of DSBs and to the prevention of gross chromosomal alterations, such as those observed in cancer cells (Figure 2).[21,22]

Taking into account the above described functions of H2AX in DNA repair and DNA stability maintenance, the use of gH2AX as a biomarker for early cancer detection and prognosis has been proposed.

Methods of gH2AX detection

The main techniques to measure gH2AX levels and kinetics are (a) immunostaining, (b) flow cytometry, (c) Western blot, and (d) enzyme linked immunosorbent assay (ELISA), with the first two methods being most commonly used, despite the fact that the last two provide an objective quantification of gH2AX levels.[23–26] Antibodies against phosphorylated serine 139 have been developed which can be useful for the identification of gH2AX foci through immunochemistry. In addition, this method can identify the kinetics of repair over a 24 h period by revealing the cellular differences in DNA DSB repair.[27,28] This technique is sensitive and specific. Flow cytometry is a novel method that allows the acquisition of cellular images on multiple channels simultaneously, providing the advantage of rapid detection of gH2AX signal in cells within different cell cycle phases, giving information on both the number and size of gH2AX foci in the nucleus.[29–31] With this technique, a favorable statistical analysis, through the quantification in high cell numbers, can be achieved. The major limitation of this method is the low resolution and, as a result, the low accuracy. The use of supportive applications such as multiparameter scanning and extended depth of field (EDF) function contribute to the

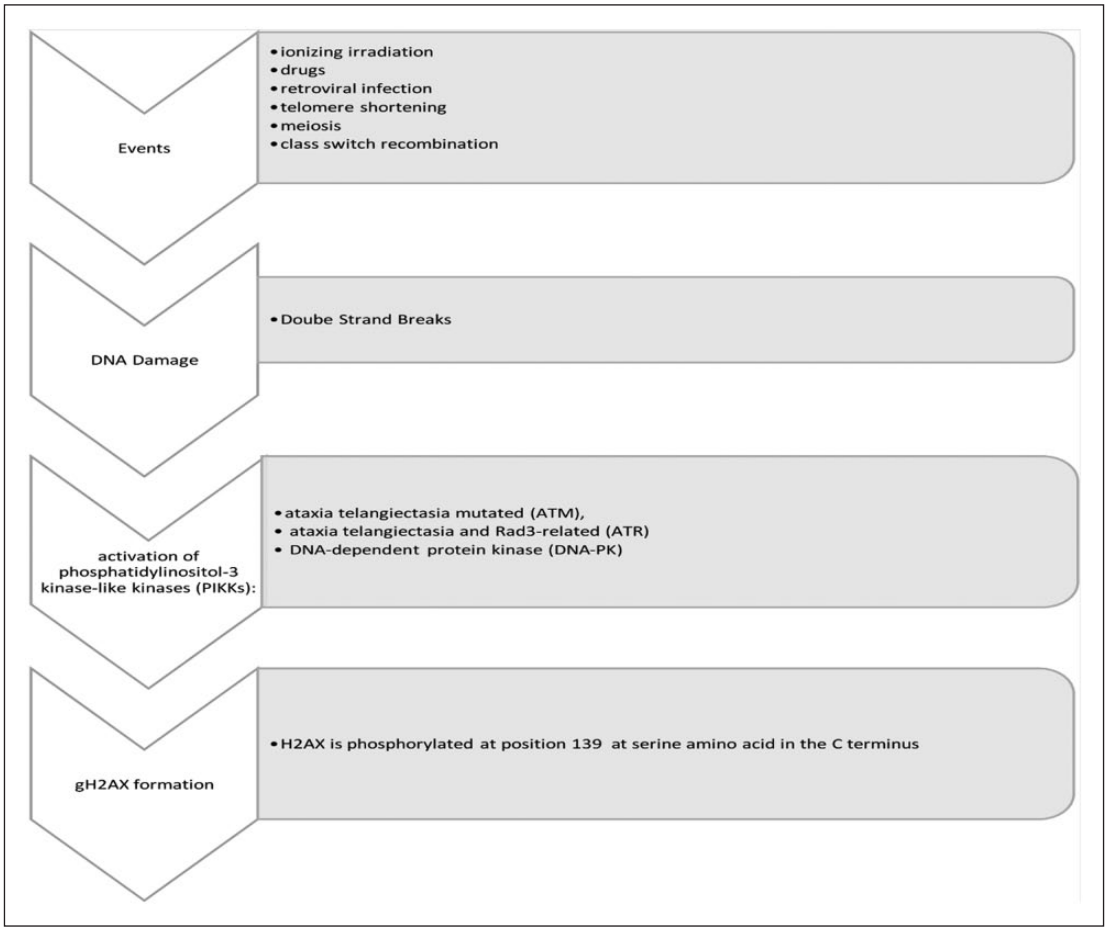

Figure 1. Sequence of events involved in gH2AX formation.

achievement of relatively high sensitivity and accuracy in determining H2AX phosphorylation in individual cells, simultaneously correlating the gH2AX expression in cell populations with DNA content or induction of apoptosis. The combination of flow cytometry with 60× magnification and EDF produced the same levels of resolution of the foci when compared to in situ foci quantitation. Compared with flow cytometry, in situ microscopy methods showed a 25%–30% increase in foci resolution.[32–34] The measurement of gH2AX immunostaining using a high-content imaging platform in chicken DT40 B-lymphocyte cell was also described by Nishihara et al.[35] Western blot has also been used to detect gH2AX foci, mainly in basic research. It is less practical for clinical studies, since the isolation of a large number of cells is demanded in order to obtain an adequate amount of nuclear proteins. ELISA is a technique that provides an objective quantification of gH2AX based on electrochemiluminescence.[36] The above described methods are used for the in vitro gH2AX detection. Cornelissen et al.[37] have found a method for the in vivo measurement. More specifically, fluorophore- and radioisotope-labeled immunoconjugates targeting gH2AX have been produced. Either diethylenetriaminepentaacetic acid (DTPA), to allow (111)In-labeling, or fluorophore Cy3 with the cell-penetrating peptide Tat, which made up the gH2AX-specific immunoconjugate, was administered intravenously to irradiated, tumor carrying mice or mice under chemotherapy. Single-photon emission computed tomography (SPECT) was conducted and radioactivity was measured, and in this way the concentration of gH2AX was also measured in vivo.[37]

gH2AX studies: biosampling

Most cell and tissue types are suitable for gH2AX detection. Variable gH2AX basal levels are found in different cell and tissue types, so that the sample type must be carefully considered in the study design.

Studies including tumor tissues obtain samples by surgery or biopsy. The collection of the samples may be easy when tumor is easily accessible as in case of biopsy or when the tumor is removed by curative surgery. On the contrary, tumors deeply located or inoperable cancers make the collection of samples more complicated. Study bias may arise due to tumor heterogeneity, differences in tumor vascularization, and necrosis. The above factors

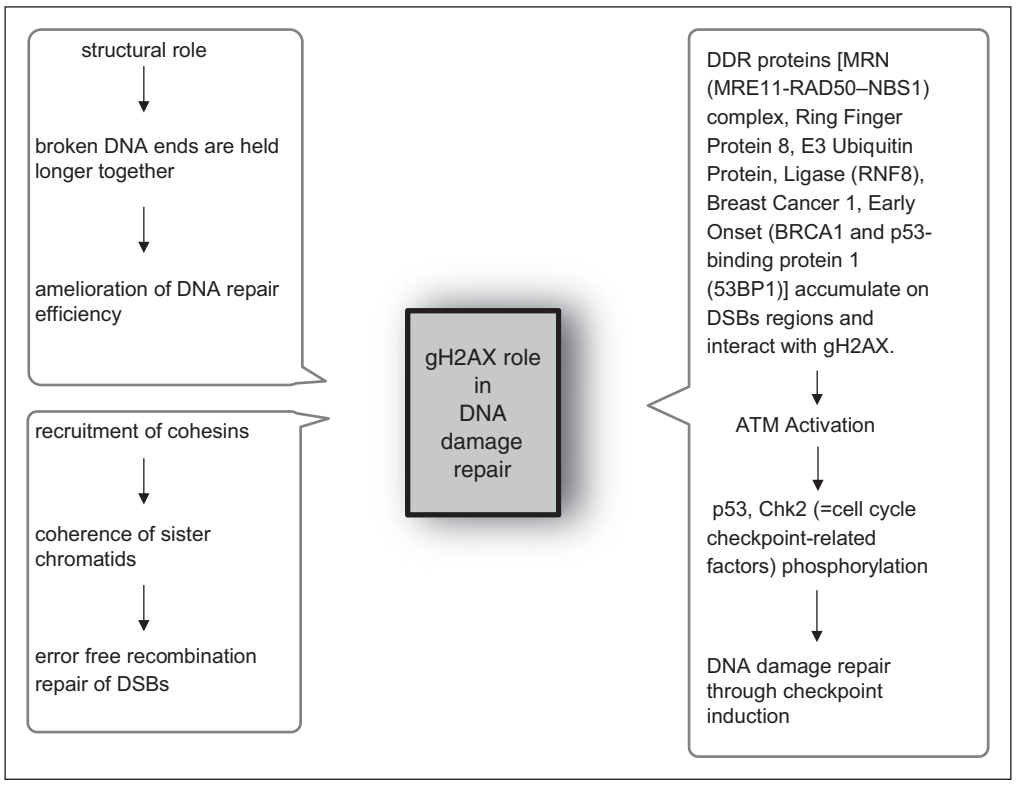

Figure 2. Role of gH2AX in DNA damage repair.

influence the metabolic and differentiation status of the cells obtained.[38] Circulating tumor cells (CTC) are an alternative to tumor tissues, offering an option for the evaluation of cancer treatments, since an increase in gH2AX foci in CTCs is observed after treatment. However, it is technically demanding as the number of cells isolated in the bloodstream is usually low or even not detectable.[39,40]

When the gH2AX kinetics are under investigation, normal cells such as lymphocytes, skin cells, plucked cells, and oral cells may be used. As far as lymphocytes' sampling is concerned, this method is easy. However, the diversity of this cell population makes the assessment of gH2AX in nonsynchronized populations problematic.[41,42] Skin and plucked cells are also easily collectable.[43] Buccal cells are easily collected and may offer a sensitive index for low-dose radiation exposure. However, the high stage of terminal differentiation and the very high background levels of DNA damage make the study of oral cells disadvantageous.[44,45]

gH2AX as a prognostic marker in several cancer types

DNA damage and genome stability are well-recognized factors associated with the progression of normal tissues to premalignant and further to malignant states. gH2AX, a marker of genomic instability, could function as a marker for cancer formation and progression. In addition, H2AX gene is located in a chromosome region (11q23), where mutations or deletions occur in a variety of human cancers.[46]

Regarding breast cancer, gH2AX is associated with the classical prognostic factors, triple negativity and BRCA1 and p53 mutations. More specifically, an elevated number of gH2AX foci in triple-negative breast cancer patients correlates with a significantly worse prognosis. After a multivariate analysis, including tumor size, grade, and triple negativity, only the interaction between triple negativity and gH2AX remained significant ($p = 0.002$).[47,48] In breast cancer patients, gH2AX was also found to be related with shorter telomeres, which was in turn associated with poorer prognosis of triple-negative breast cancer patients. This finding may offer a specific target for therapy for triple-negative breast cancer patients.[49] Similar studies were conducted with hepatic tissues and revealed that gH2AX was increased in the preneoplastic lesions of hepatocellular carcinoma and that gH2AX might be a useful biomarker in predicting the risk of hepatocellular carcinoma. More specifically, gH2AX was significantly increased in nontumorous tissues of hepatocellular carcinoma as compared with liver cirrhosis without hepatocellular carcinoma ($p < 0.005$).[50] Mei et al.[51] investigated the expression of gH2AX in ovarian cancer patients and concluded that gH2AX may be used to detect epithelial ovarian cancer at an early stage and to identify the subgroup of patients who are more susceptible to relapse. The expression of gH2AX was found to be higher in epithelial ovarian cancer tissues

than in normal tissues. Moreover, among resistant cases, high gH2AX expression rates were found in 80%, whereas these rates were 53.2% among sensitive cases ($p = 0.025$). Elevated gH2AX was correlated with shorter disease-free survival, whereas overall survival was not influenced.[51] Similar results have been reported by a study on human melanoma cells.[52,53] Melanoma cells (in situ, primary cutaneous, and metastatic) showed a statistically higher gH2AX expression in comparison to cells derived from nevus (benign, dysplastic, and Spitz), although this expression was not correlated to a variety of melanoma prognostic factors, such as Breslow depth, mitotic rate, and sentinel lymph node status.[52,53] Burns et al.[54] investigated the potential quantitative link between rat skin cancer induction and gH2AX foci in rat keratinocytes exposed to radiation in vitro. The authors revealed that cancer yields per rat were consistently predictable based on gH2AX foci yields in keratinocytes in vitro.[54] The overexpression of gH2AX in cervical intraepithelial neoplasia (CIN) II and III and in invasive squamous cell carcinoma (ISCC) reveals its potential role in malignant transformation of cervical squamous epithelial cells.[55] The authors also suggested the involvement of gH2AX in different biological mechanisms as an early event leading to resistance to apoptosis in vulvar carcinogenesis.[56] Carlson et al.[57] indicate that gH2AX is overexpressed in lichen sclerosus of the vulva/penis and squamous cell carcinoma. Brunner et al.[58] investigated the role of gH2AX in endometrial tumorigenesis. Increased staining levels are positively related to classical prognostic factors, such as tumor stage, tumor grade, histological type, vascular space involvement, as well as shortened disease-free and overall survival. The authors indicate that gH2AX could potentially be useful as an additional histopathological prognostic factor in patients with endometrial cancer.[58] Another study revealed that expression of gH2AX detected by immunohistochemistry may represent an independent prognostic indicator of overall survival in patients with non–small cell lung cancer, with a 2.15-fold increase in risk of death in individuals with high expression of gH2AX.[59] Ibuki et al.[60] showed that gH2AX is a sensitive marker of DNA adducts and provides a possible system for genotoxicity screening of chemicals such as NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone), which need metabolic activation to induce DNA damage and which in this case is responsible for lung cancer. In addition, gH2AX expression by oral squamous cell carcinoma cells is associated with reduced overall survival time, independent of other variables in patients.[61] Moreover, elevated gH2AX levels were detected in colon carcinomas, fibrosarcoma, osteosarcoma, glioma, and neuroblastoma cells.[62] In addition, increased gH2AX contents were found in colonocytes from ulcerative colitis patients, a chronic inflammatory disease that predisposes patients to colorectal cancer.[63] Lee et al.[64] revealed that gH2AX was correlated with a more malignant cancer behavior as well as a poor patient survival indicating that gH2AX may be an independent prognostic predictor for colorectal cancer. gH2AX was also investigated in patients with larynx cancer and it was shown to be an independent prognostic factor, remaining significant when clinical findings such as tumor localization, tumor stage, smoking, and alcohol habits were controlled. In addition, when measured as a continuous variable, gH2AX was shown to have a significant positive influence with better laryngoesophageal dysfunction–free survival, although this was not significant for overall survival.[65] Fernández et al.[66] investigated the levels of gH2AX in peripheral blood lymphocytes (PBL) in patients with bladder cancer. A significant association between mutagen-induced gH2AX and occurrence of bladder cancer was found which was irrespective of age, sex, and smoking status.[66] In another study, gH2AX positivity was found to be associated with a lower rate of tumor recurrence in urothelial cancer patients, suggesting the prevention of accumulation of genomic damage.[67] In addition, Toyoda et al.[68] suggested that gH2AX may be a potential biomarker for the early detection of genotoxic bladder carcinogens. In another study, gH2AX was found to play a role in the differentiation status of thyroid cancer.[69]

Table 1 summarized the main studies, which analyzed the use of gH2AX as a prognostic tool in several cancer types.

Taking the above mentioned studies in consideration, it could be suggested that gH2AX has a potential role in carcinogenesis and in early cancer diagnosis. This is the reason why its prognostic role has to be further investigated.

Conclusion

The incidence cancer is expanding worldwide with catastrophical consequences for the patients and their families. The whole medical community aims to find diagnostic and therapeutical tools in order to make a diagnosis at early stages so as to avoid the adverse effects of complicated therapeutic schemata.

Genomic instability and DSBs are presuppositions for the derivation of tissue cells from the normal cell cycle and the development of cancer. The medical community, in the last years, has concentrated on the detection of markers that are involved in the early steps of cancer development. Among these markers is gH2AX, which has already been studied in a variety of cancers and precancerous lesions. gH2AX detection provides a considerably more sensitive, efficient, and reproducible measurement of the amount of DNA damage compared to other techniques, such as pulsed field gel electrophoresis and comet assays. It has been found that elevated levels of gH2AX are present in a number of human cancer model systems, including cervical cancer cells, melanoma cells, colon carcinomas, fibrosarcoma, osteosarcoma, glioma, and neuroblastoma cells.[80]

Table 1. gH2AX in several cancer types.

Author	Year	Materials	Methods	Conclusions
Bhatia et al.[70]	2013	Fine needle aspirates from patients with IDC and FA	Flow cytometry	Increased presence of spontaneous gH2AX foci in IDC as compared to FA was observed. A higher expression of gH2AX in diploid IDCs may explain the genetically stable nature of these lesions and may serve as a means of assigning the patients of IDC to a better prognostic category.
Brunner et al.[58]	2011	Paraffin-embedded tissue blocks from EC patients	IHC	gH2AX staining was significantly associated with advanced tumor stage ($p = 0.04$), histological grade ($p < 0.0001$), histological type ($p < 0.0001$), and vascular space involvement ($p = 0.05$), but not with lymph node involvement ($p = 0.64$) and patients' age ($p = 0.36$). gH2AX staining was associated with a shortened disease-free and overall survival. A strong positive correlation was observed between gH2AX and p53 expression ($p < 0.0001$). Increased expression levels of gH2AX are associated with unfavorable prognostic factors in type I and type II endometrial carcinomas.
Brustmann et al.[55]	2011	Tumor samples: NE CIN cervical ISCC	IHC	gH2AX expression increased from NE and CIN1 to CIN2/3 ($p = 0.0001$). Overexpression of gH2AX in CIN2/3 and ISCC of the uterine cervix reflects the neoplastic transformation of cervical squamous epithelia in reaction. Increased DNA damage.
Brustmann et al.[56]	2011	Formalin-fixed, paraffin-embedded archival vulvar tissues NE CIN cervical ISCC	IHC	Immunoscores did not differ between grade 1 and grade 2/3 intraepithelial neoplasia. gH2AX nuclear surviving expression increased from NE and lichen sclerosus to high-grade classic vulvar intraepithelial neoplasia, differentiated vulvar intraepithelial neoplasia, and ISCC, significantly ($p = 0.0001$), and followed the distribution of immature squamous epithelial cells.
Carlson et al.[57]	2013	Tissue samples Lichen sclerosus of the vulva/penis Squamous cell carcinoma	IHC	Overexpression of gH2AX in lichen sclerosus of the vulva/penis and squamous cell carcinoma.
Cheung et al.[67]	2009	Tissue samples from patients with low-grade bladder urothelial carcinoma	IHC	Significant association between mutagen-induced gH2AX and occurrence of bladder cancer.
De Miguel-Luken et al.[65]	2016	Larynx tumor samples	IHC	gH2AX in patients with larynx cancer was shown to be an independent prognostic factor, remaining significant when clinical findings such as tumor localization, tumor stage, smoking, and alcohol habits were studied.
Fernández et al.[66]	2013	PBL	Novel, image-based, high-throughput phenotypic assay (iCys™ laser scanning cytometer).	Significant association between mutagen-induced gH2AX and occurrence of bladder cancer, irrespective of age, sex, and smoking status.
Guo et al.[71]	2015	Tissue samples from patients with superficial gastritis atrophic gastritis gastric carcinoma	IHC	A significant increase in gH2AX expression was found between gastritis and gastric carcinoma. No significant relation with patient's age, depth of invasion, lymph node metastasis, or the tumor-node-metastasis stage of the gastric carcinoma.
He et al.[72]	2013	PBL from patients with lung cancer	Laser scanning cytometer-based immunocytochemical method	High gH2AX ratio was significantly associated with increased risk of lung cancer. gH2AX may be a novel phenotypic marker of lung cancer risk.

(Continued)

Table 1. (Continued)

Author	Year	Materials	Methods	Conclusions
Hu et al.[69]	2014	Tissue samples Benign nodular goiter Well differentiated thyroid cancer Poorly differentiated thyroid cancer Anaplastic thyroid cancer	IHC	gH2AX expressed higher in thyroid cancer tissues than in benign nodular goiter and normal adjacent tissues. gH2AX expression was associated with FT3. gH2AX was also associated with T classification, TNM stage, FT4, TSH, and differentiation status. gH2AX was correlated with ATM in thyroid cancer.
Kim et al.[73]	2010	Tissue samples from gastric adenoma and gastric adenocarcinoma	IHC	In gastric carcinoma cells, gH2AX was highly expressed as compared to normal epithelial cells and gastric adenoma ($p < 0.01$). No differences in the expression of gH2AX between normal epithelium and gastric adenoma. The expression of gH2AX was not significantly different according to the clinicopathological parameters in the patients with gastric adenocarcinoma.
Lee et al.[64]	2015	Colorectal cancer tissue samples	IHC	gH2AX was correlated with a more malignant cancer behavior as well as a poor patient survival. gH2AX may be an independent prognostic predictor for colorectal cancer.
Matthaios et al.[59]	2013	Paraffin-embedded specimens of non–small cell lung cancer	IHC	High expression of gH2AX remained an independent prognostic factor of shorter overall survival. p53 low/gH2AX low phenotype was associated with significantly better survival compared with other phenotypes.
Matsuda et al.[50]	2013	Tissue samples HCC Dysplastic nodule Nontumorous liver diseases	IHC Fluorescence analysis Western blotting	All cases with chronic liver disease showed increased levels of gH2AX expression. gH2AX was significantly increased in nontumorous tissues of HCC as compared with liver cirrhosis without HCC. gH2AX was increased in the preneoplastic lesions of HCC and might be a useful biomarker in predicting the risk of HCC.
Mei et al.[51]	2015	Tissue samples EOC Normal ovarian tissues	IHC	The expression levels of gH2AX protein in EOC were higher than that in normal tissues ($p < 0.001$). gH2AX expression was not significantly correlated with age, Histopathological type, tumor differentiation, lymph node metastasis, or FIGO stages. DFI was negatively correlated with the gH2AX expression, where higher expression of gH2AX resulted in shorter DFI while no overall survival difference was detected. gH2AX may be used to detect EOC at an early stage and identify women at higher risk for relapse.
Mhawech-Fauceglia et al.[74]	2014	EC tissue samples	IHC	In EC type I, gH2AX positivity and with advanced tumor stage, the presence of lymphovascular invasion and tumor recurrence.
Nagelkerke et al.[47]	2011	Formalin-fixed, paraffin-embedded breast cancer cell lines and tumors	IHC	gH2AX positivity was associated with the triple negative/basal-like subgroup ($p = 0.005$), and with BRCA1 ($p = 0.011$) or p53 ($p = 0.053$) mutations. Constitutive gH2AX and p53 staining reveals a subset of patients with triple-negative breast tumors that have a significantly poorer prognosis. In a multivariate analysis with tumor size, grade, and triple negativity, only the interaction between triple negativity and gH2AX remained significant.
Oliveira-Costa et al.[61]	2014	OSCC	IHC	gH2AX expression by OSCC cells are associated with reduced overall survival time, independent of other variables in patients, as well as gender.
Risques et al.[63]	2008	Tissue samples from patients with ulcerative colitis	IHC	gH2AX intensity is higher in colonocytes of ulcerative colitis patients, which might explain the increased and earlier risk of cancer in this disease.

(Continued)

Table 1. (Continued)

Author	Year	Materials	Methods	Conclusions
Sedelnikova et al.[62]	2011	Colon, breast, ovary, liver, and kidney tumors and normal adjacent tissues from cancer patients	IHC	Elevated endogenous levels of gH2AX in various human cancer cell lines, premalignant lesions, and solid tumors.
Turinetto et al.[75]	2016	Peripheral blood mononuclear cells before and after their exposure to IR		Patients with high basal H2AX phosphorylation levels had a decreased risk of recurrence or progression.
Warters et al.[53]	2005	Discarded human neonatal foreskins Melanoma cell lines	Flow cytometry Immunofluorescence microscopy Western blotting	Expressed levels of gH2AX foci in melanoma cells.
Wasco et al.[52]	2008	Melanocytic lesions	Immunofluorescence microscopy	gH2AX has a possible role in melanomagenesis. gH2AX was observed at higher levels (percentage and intensity of staining) in melanoma in situ, primary cutaneous melanoma (with the exception of desmoplastic melanoma), and metastatic melanoma, which was statistically different from that in benign nevus, dysplastic nevus, and Spitz nevus considered together ($p < .0001$). The expression of gH2AX did not show significant correlation with many melanoma prognostic factors, including Breslow depth, mitotic rate, and sentinel lymph node status.
Xiao et al.[76]	2015	HCC tumor samples	IHC	Increased gH2AX expression in HCC is associated with tumor size, vascular invasion, TNM stage, and reduced survival rate after liver transplantation. gH2AX is associated with angiogenesis of HCC. gH2AX or a combination of gH2AX/EGFR/HIF-1α is a novel marker in the prognosis of HCC after liver transplantation and a potential therapeutic target.
Xie et al.[77]	2014	GC tissue samples	Immunofluorescence microscopy Western blotting	Overexpression of gH2AX in GC was found to correlate with tumor location, gross appearance, differentiation, depth of invasion, TNM stage, and lymph node metastasis. Expression of gH2AX may serve as a valuable biomarker for the diagnosis and progression of GC.
Xu et al.[78]	2013	PBL from patients with esophageal adenocarcinoma	Laser scanning cytometer-based immunocytochemical method	Radiation-induced DNA damage assessed by gH2AX ratio is associated with an increased risk of esophageal adenocarcinoma.
Yu et al.[80]	2006	Cell lines (breast, ovary, melanoma, lung, colon, and central nervous system)	Immunofluorescence	Higher gH2AX levels in ulcerative colitis, colon cancer, fibrosarcoma, osteosarcoma, glioma, and neuroblastoma cells. Higher gH2AX rates detected in cancer cells.
Zhang et al.[79]	2014	LSCC	Immunofluorescence	gH2AX expression was frequently detected in LSCC tissues ($p < 0.001$). Not associated with any positive clinicopathological characteristics. Nuclear gH2AX expression was an independent risk factor for the overall survival. Expression of nuclear gH2AX might be closely associated with the prognosis of LSCC.

IDC: infiltrating ductal carcinoma; FA: fibroadenoma; EC: endometrial cancer; IHC: Immunohistochemistry; NE: normal squamous cervical epithelia; CIN: cervical intraepithelial neoplasia; ISCC: invasive squamous cell carcinoma; PBL: peripheral blood lymphocytes; FT3: free triiodothyronine; TNM: tumor, node, metastasis; FT4: free thyroxine; TSH: thyroid-stimulating hormone; ATM: ataxia telangiectasia mutated; HCC: hepatocellular carcinoma; EOC: epithelial ovarian cancer; FIGO: International Federation of Gynecology and Obstetrics; DFI: disease-free interval; OSCC: oral squamous cell carcinoma tissue samples; IR: ionizing radiation; EGFR: epidermal growth factor receptor; HIF-1α: hypoxia inducible factor 1α; GC: gastric cancer; LSCC: laryngeal squamous cell carcinoma.

For these reasons, detection of gH2AX through human biopsies and/or aspirates could be used for early cancer screening. Additional studies should be carried out to further investigate the prognostic role of this marker in several cancer types and to associate it with the established tumor markers.

Declaration of conflicting interests

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