Submitted: 20 September, 2023 Accepted: 20 October, 2023 Published: 15 March, 2025 DOI:10.22514/ejgo.2025.032

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

The role of γH2AX and H2AX in cervical carcinogenesis, invasion, and metastasis, and their relationship with the expression of E-cadherin

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Abstract

Background: Our previous studies found a link between increased γH2AX expression and cervical carcinogenesis. However, the roles and relationships of γH2AX and its precursor protein, H2AX, in carcinogenesis, invasion, and metastasis of cervical cancer, as well as their association with epithelial-mesenchymal transition (EMT), remain unclear. **Methods**: The expression of H2AX, γH2AX and the EMT marker E-cadherin was assessed through immunohistochemical staining in 66 cases of cervical intraepithelial neoplasia (CIN) and 87 cases of primary squamous cell carcinoma (SCC). Furthermore, the expression of H2AX, γH2AX and E-cadherin was evaluated in tumor budding (78 patients), tumor emboli (18 patients), or lymph node metastases (16 patients), along with their corresponding central tumors in the same SCC patients. Lastly, the correlation between the expression of γH2AX and H2AX in central tumors, tumor budding, and the clinicopathological characteristics of patients was investigated. **Results**: In CIN and SCC, the expression of γH2AX is upregulated as the severity of the disease, while the expression of H2AX and E-cadherin is downregulated. In tumor budding, tumor emboli, and metastatic lymph nodes, γH2AX expression is significantly higher than in the central tumor, while H2AX expression is significantly lower. The expression of E-cadherin is significantly lower in tumor budding and tumor emboli, compared to the central tumor. Conversely, the expression of E-cadherin in lymph nodes is significantly higher than in tumor budding. γH2AX expression in tumor budding is associated with FIGO staging, tumor emboli, and menopausal status. **Conclusions**: γH2AX and H2AX play significant roles in the processes of carcinogenesis, invasion, and metastasis in cervical cancer. Moreover, H2AX may potentially promote cervical cancer invasion and metastasis through its involvement in the EMT, while γH2AX does not participate in this process. **Clnical Trial Registration**: ChiCTR-TRC-1800016405.

Keywords

Cervical squamous cell carcinoma; Cervical intraepithelial neoplasia; Phosphorylated H2AX; Histone H2AX; EMT; DNA double-strand damage

1. Introduction

Cervical cancer is the most common gynecological cancer in the world. In 2020, there were approximately 604,127 new cases of cervical cancer and 341,831 deaths from the disease worldwide, accounting for 6.5 and 7.7% of new cases and deaths in women, respectively [1]. In China, there are approximately 112,000 new cases of cervical cancer and 61,579 deaths from the disease each year [2].

H2AX, a variant of the core histone H2A, is known to play an important role in the repair of DNA double-strand breaks (DSBs) and genome stability. H2AX is phosphorylated at the N-terminal serine 139 in response to DNA damage in cells (referred to as phosphorylated H2AX (γH2AX)). Furthermore, the presence of γH2AX at DSBs and at breakpoints in chromosomes is known to facilitate the assembly of proteins for DNA repair. Consequently, γH2AX is widely used as a marker for detecting DSBs [3]. In addition, γH2AX is recognized as a potential marker for the initiation and progression of cancer [4]. High expression levels of γH2AX were previously detected in various types of cancer when compared to normal tissues [5–7], and also associated with the prognosis and treatment responses of patients with certain types of cancer [8, 9]. Our laboratory previously demonstrated that the overexpression of γH2AX in cervical intraepithelial neoplasia CIN 1, CIN 2/3, and squamous cell carcinoma (SCC), reflects the transformation of the cervical squamous epithelium after increased DNA damage [10]. In addition to its central role in the response to DNA damage, H2AX is also known to exhibit an oncogenic role in cancer [3, 11].

Some studies have found that H2AX is a potential regulator of epithelial mesenchymal transition (EMT) [12, 13]. EMT is a process in which cells lose their epithelial properties and gain their mesenchymal properties. Furthermore, EMT is a key event in the morphological transformation of cancer cells and contributes to their malignant biological properties, including invasion and metastasis. A loss in the expression of the adhesion junction protein E-cadherin is an established hallmark of EMT, and is thought to enable metastasis by disrupting intercellular contact, an early step in metastatic dissemination.

During metastasis, cancer cells with metastatic potential must first detach from the primary tumor and intravasate into the lymphovascular system, travel and survive within the circulatory system, and extravasate from the blood vessels to attach to the target organ, finally developing into new lesions. Thus, from a morphological point-of-view, the metastatic process of cervical cancer may consist of four phases: tumor bud formation, lymphovascular invasion, lymphovascular space invasion (tumor emboli formation), and regional lymph node and/or distant metastasis. This process involves trans-differentiation processes in tumor cells such as EMT and mesenchymal epithelial transition (MET, the reverse transformation of mesenchymal cells to epithelial cells and the reacquisition of epithelial markers; this represents the basis for the colonization of tumor cells to distant secondary sites). Tumor budding, defined as 'single tumor cells or clusters of less than five tumor cells at the invasive front of the tumor' [14, 15], is considered the first phase of invasion and metastasis, and often used as a histomorphological marker of aggressive tumor behavior [14, 16]. Furthermore, tumor budding is considered a morphological manifestation of EMT [14, 15, 17].

Research investigating the roles of γH2AX and H2AX in the initiation, invasion and metastasis of cervical cancer, and their association with E-cadherin, a marker of EMT, is limited. Consequently, there is a need to investigate the interplay between these factors during the progression of cervical cancer. In this study, we investigated the expression levels of H2AX, γH2AX and E-Cadherin in CIN and SCC by immunohistochemistry. Considering the spatial distribution characteristics during the invasion and metastasis of cervical cancer, we further evaluated their expression levels in central tumors, tumor budding, tumor emboli and metastatic lymph nodes. We also correlated their expression levels in central tumors and tumor budding with the clinicopathological characteristics of patients. Our results suggest that γH2AX and H2AX play significant roles in the processes of carcinogenesis, invasion and metastasis in cervical cancer. Moreover, H2AX may potentially promote the invasion and metastasis of cervical cancer via its involvement in EMT; however, γH2AX does not participate in this process.

2. Materials and methods

2.1 Sample collection

We enrolled participants who satisfied specific inclusion criteria: a confirmed histological diagnosis of either cervical cancer or cervical intraepithelial neoplasia (CIN). Participants were excluded if they had received any prior treatments for cervical diseases, including loop electrosurgical excision procedure (LEEP), cold-knife conization, cryotherapy, laser therapy, hysterectomy, chemotherapy or radiation therapy for cervical neoplasia. Additional exclusion criteria included pregnancy, Human Immunodeficiency Virus (HIV) infection and a current or past history of other malignant diseases. Using these criteria, we collected cervical tissue samples from a total of 153 patients at the Pathology Department of Gansu Provincial Cancer Hospital between 2014 and 2018. Of the samples obtained, 66 samples were obtained from patients who had undergone cervical conization or biopsy for cervical intraepithelial neoplasia (CIN), while the remaining 87 samples were acquired from patients who had undergone radical abdominal hysterectomy for squamous cell carcinoma (SCC). Each patient contributed one tissue block from the lesion, and patients with SCC complicated by lymph node metastasis contributed another tissue block of lymph node metastasis. If tumor budding or tumor emboli were available, tumor budding and their paired central tumors or tumor emboli, and their paired central tumors and tumor budding, were evaluated separately. The final histological diagnoses were as follows: CIN 1 corresponding to low-grade squamous intraepithelial lesions (n = 32), CIN 2/3 corresponding to high-grade squamous intraepithelial lesions (n = 34), SCC (n = 87; 78 SCC patients had tumor budding, of which 18 had tumor emboli and 16 had lymph node metastasis). The specimens were fixed in formalin and embedded in paraffin. Formalin fixation did not exceed 24 h. All hematoxylin and eosin-stained sections and clinical histories were reviewed.

2.2 Immunohistochemical staining

Formalin-fixed and paraffin-embedded sections with a thickness of 4 μm were mounted on poly-L-lysine-coated slides (Maixin Biotechnology, Fuzhou, China). The sections were deparaffinized in xylene and rehydrated through a series of graded ethanol solutions. Immunohistochemical staining was performed using an automatic immunostainer (Autostainer-Link 48, Agilent Technologies, Santa Clara, CA, USA) in accordance with the manufacturer's instructions. The primary antibodies were as follows: anti-Ser139 phosphorylated H2AX (cat. no. 05–636; mouse monoclonal antibody; 1:200; Millipore, Billerica, MA, USA), anti-E-cadherin (cat. no. MAB-0589; mouse monoclonal antibody; 1:100; Maixin Biotechnology) and anti-histone H2AX (cat. no. sc-517336; mouse monoclonal antibody; 1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Positive controls (*i.e.*, sections of tissues known to express the relevant antigen) and negative controls (*i.e.*, duplicate sections processed as above, except that primary antibodies were omitted) were also included in this experiment.

2.3 Analysis of immunohistochemical staining

All sections were independently analyzed by two experienced pathologists. Cancer cells exhibiting nuclear staining, regardless of the presence of cytoplasmic staining, were considered as being positively immunostained for γH2AX and H2AX, and those showing cell membrane staining were considered

as positively immunostained for E-cadherin. The expression levels of γH2AX, H2AX and E-cadherin were represented as the percentage of positive cells and the intensity of immunostaining. The percentage scores were as follows: 0 (none); 1 (≤10%); 2 (11–25%); 3 (26–50%); 4 (51–75%), and 5 (≥76%). The intensity scores represented the average staining intensity of positive tumor cells, as follows: 1 for weak, 2 for intermediate, and 3 for strong. We evaluated five fields at high-power (×400 magnification) for each specimen. The average intensity scores and the average percentage scores were multiplied to obtain the total histoscore (H score) (range = 0–15) [18].

If tumor budding was present in a specimen of SCC, it was first estimated in each patient by an experienced pathologist on HE-stained sections according to guidelines published by the International Tumor Budding Consensus Conference [19]. Firstly, a hotspot area was identified at the front of tumor infiltration using a 20× microscope objective. Secondly, tumor buds in the selected area were counted under a 40× microscope objective. Cases were then divided into two groups: a low budding group (<10 buds) and a high-budding group (≥10 buds).

For tumors in which tumor budding was present, two regions of that tumor (the central tumor and the tumor budding) were selected and the expression levels of γH2AX, H2AX and E-cadherin were determined. With regards to the central tumor, which was considered to be the largest extended tumor area, at least five fields in each section were randomly selected and analyzed. Tumor budding, defined as a single cell or a cluster of up to five cells isolated from the central tumor that broadly infiltrated the adjacent stroma, were evaluated in five randomly selected fields, or in each field if there were fewer than five tumor budding fields. The evaluation of tumor emboli was performed according to previously published methodology for the assessment of tumor budding [20].

2.4 Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (IBM, Armonk, NY, USA). The Student's *t*-test, analysis of variance (ANOVA) or Kruskal-Wallis non-parametric test was used to identify differences between groups. Pearson's correlation analysis was applied for correlation analysis. Results were considered to be statistically significant at $p < 0.05$.

3. Results

3.1 In CIN and cancer cases, the expression levels of γH2AX were upregulated with increasing disease severity; in contrast, the expression levels of H2AX and E-cadherin were downregulated

Immunohistochemical staining showed that γH2AX was located in the nuclei of parenchymal cells of cervical cancer and CIN, H2AX was located in the nuclei of parenchymal cells as well as in surrounding stromal cells, and E-cadherin was located in the parenchymal cell membrane (Fig. 1A). As shown in Fig. 1A,B, γH2AX expression was significantly higher in CIN 2/3 compared to CIN 1 ($p < 0.05$, Fig. 1A), and significantly higher in SCC compared to CIN 2/3 ($p < 0.01$). In contrast, the expression of H2AX and E-cadherin was lower in SCC compared to CIN 2/3 ($p < 0.05$ and $p < 0.01$, respectively), and significantly lower in CIN 2/3 compared to CIN 1 (both $p < 0.01$; Fig. 1A,C,D).

3.2 Lower levels of H2AX and E-cadherin expression and higher expression levels of γH2AX were associated with the presence of tumor budding

To understand the expression patterns of γH2AX, H2AX and E-cadherin during invasion, we examined the expression of these markers in tumor budding and central tumors of 78 cases of SCC with tumor budding. As shown in Fig. 2, the expression of γH2AX was higher in deeply invading nests (Fig. 2A). Particularly, in tumor budding (Fig. 2B), its expression was significantly higher than that in central tumors ($p < 0.05$; Fig. 2F). In contrast to the expression pattern of γH2AX, the expression of H2AX and E-cadherin was significantly reduced in tumor budding compared to central tumors (both $p < 0.01$; Fig. 2C,D,G,H). Additionally, we noticed decreased H2AX expression in spindle-shaped cells at the margins of cancer nests in some cases (Fig. 2E).

3.3 Lower H2AX and E-cadherin expression levels and higher levels of γH2AX expression were associated with tumor emboli and lymph node metastasis

To investigate the expression patterns of γH2AX, H2AX, and E-cadherin in metastasis, we analyzed 28 cases of SCC with tumor emboli based on clinicopathological data. Among these, 18 cases showed central tumors, tumor budding, and tumor emboli in all sections. Of 19 cases with metastatic lymph nodes, 16 had central tumors, tumor budding, and metastatic lymph nodes in all sections. As shown in Figs. 3,4, we compared the expression of these markers in tumor emboli and metastatic lymph nodes with central tumors and tumor budding.The results demonstrate that γH2AX expression was significantly increased in tumor emboli and metastatic lymph nodes compared to central tumors ($p < 0.05$ and $p < 0.01$, respectively; Figs. 3A,B,4A,B). In contrast, H2AX expression was markedly reduced in tumor emboli and metastatic lymph nodes (both $p < 0.01$; Figs. 3C,D,4D,E). However, no statistically significant differences in expression levels were observed among tumor emboli, metastatic lymph nodes, and tumor budding (all $p > 0.05$; Fig. 4A,B,C,D,E,F).For E-cadherin, expression was significantly decreased in tumor emboli compared to central tumors ($p < 0.05$; Figs. 3E,4G), whereas no significant difference was found between tumor emboli and tumor budding ($p > 0.05$; Fig. 4G). Notably, E-cadherin expression in metastatic lymph nodes was higher than in tumor budding ($p < 0.01$; Fig. 4H), but no significant differences were observed when comparing metastatic lymph nodes to central tumors and tumor emboli (both $p > 0.05$; Figs. 3F,4H,I).

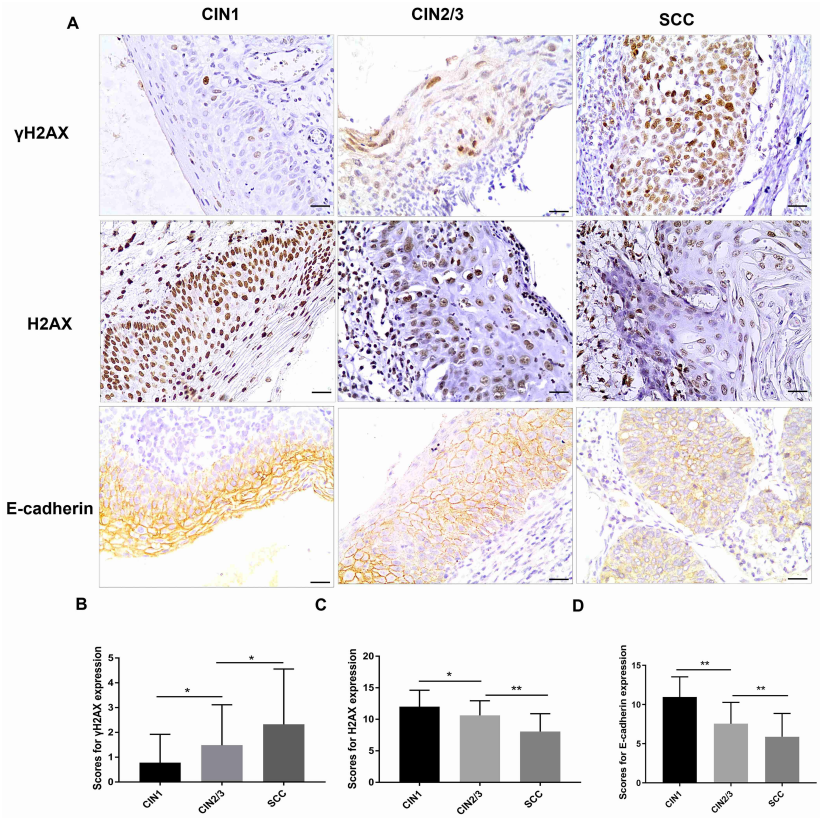

FIGURE 1. Immunohistochemical staining of γH2AX and H2AX in cervical intraepithelial neoplasia specimens. Representative stained images of γH2AX, H2AX and E-cadherin in CIN 1, CIN 2/3 and squamous cell carcinoma (SCC) specimens (A). Comparison between the expression of γH2AX (B), H2AX (C) and E-cadherin (D) in CIN 1, CIN 2/3, and SCC cases (N = 32, N = 34 and N = 87, respectively). Scale bar = 300 μm. *, $p < 0.05$; **, $p < 0.01$. CIN: cervical intraepithelial neoplasia.

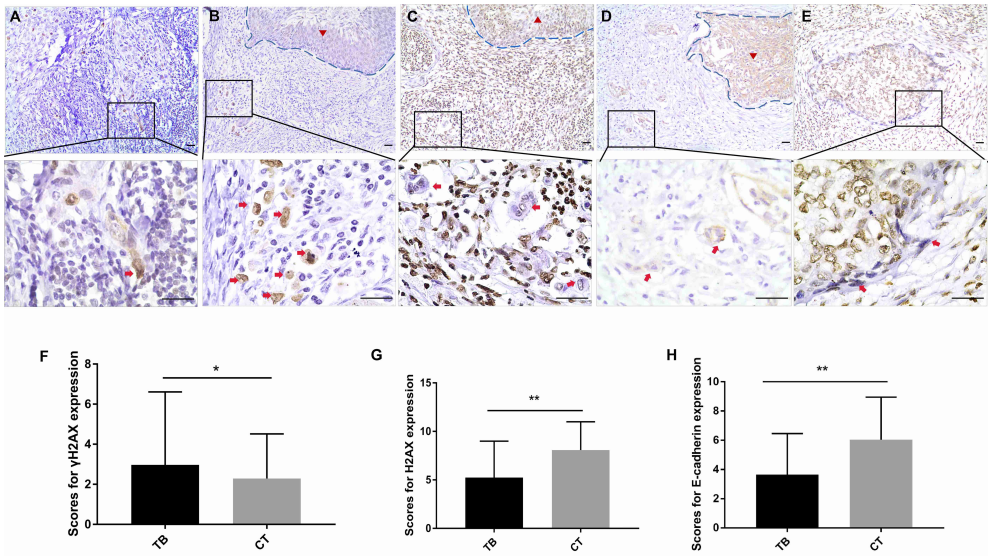

FIGURE 2. Immunohistochemical staining of γH2AX and H2AX in central tumors and tumor budding. Representative stained images of γH2AX at the front of deeply invading nests (the area indicated by the red arrow) (A). Representative stained images of γH2AX (B), H2AX(C) and E-cadherin (D) in central tumors (the area indicated by the red triangle inside the region enclosed by the blue-dotted-line) and tumor budding (the cells indicated by red arrows). Representative images of morphology and H2AX expression in cancer cells at the margins of cancer nests (the cells indicated by red arrows) (E). A comparison between the expression of γH2AX (F), H2AX (G), and E-cadherin (H) in central tumors and tumor budding in the same patients (N = 78). Scale bar = 300 μm. CT: central tumors; TB: tumor budding. *, $p < 0.05$; **, $p < 0.01$.

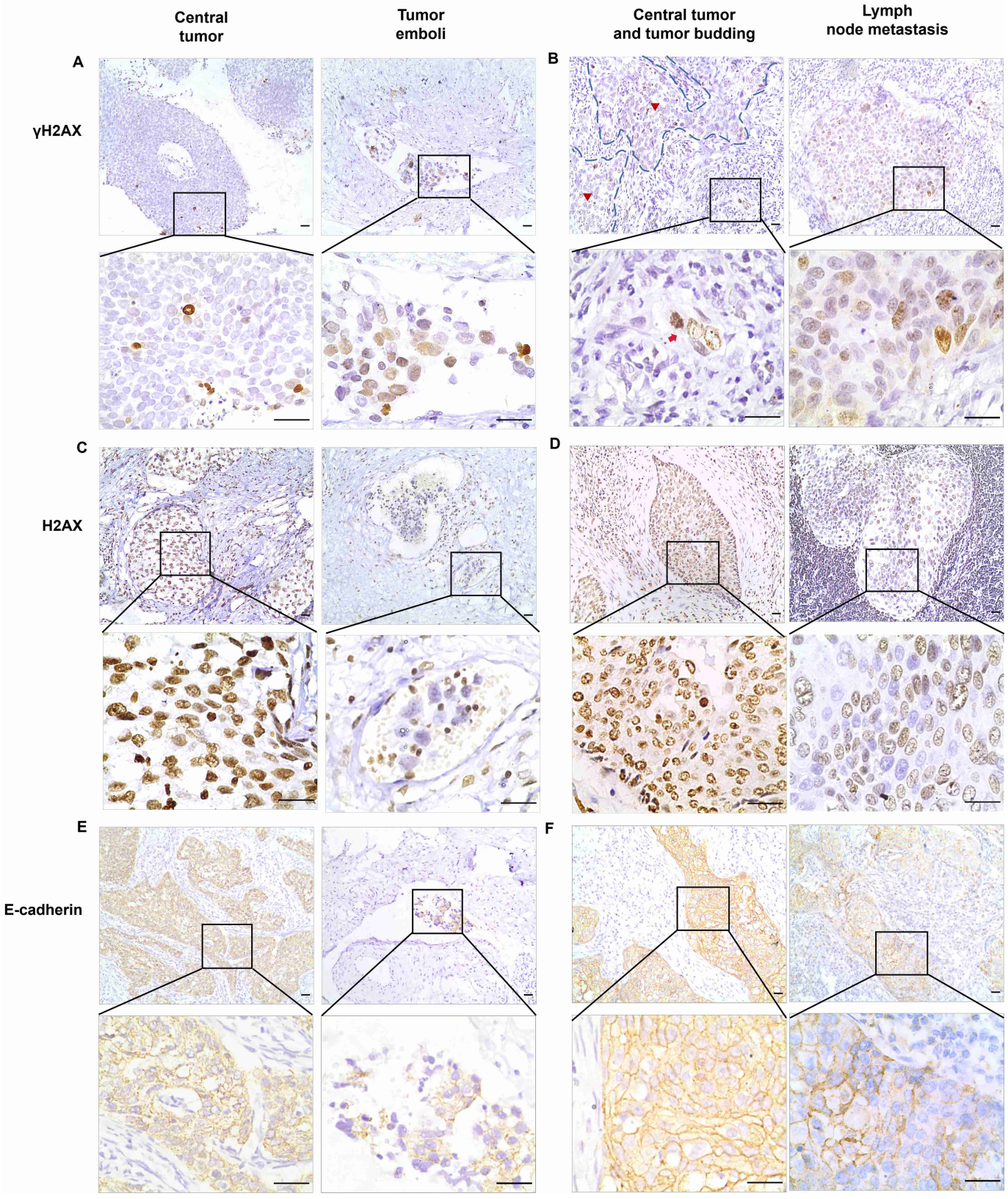

FIGURE 3. Immunohistochemical staining of γH2AX, H2AX and E-cadherin in central tumors, tumor budding, tumor emboli and metastatic lymph nodes. Representative stained images of γH2AX (A), H2AX (C) and E-cadherin (E) in central tumors and tumor emboli in the same patient. Representative stained images of γH2AX in central tumors (the area indicated by the red triangle inside the region enclosed by the blue-dotted-line), tumor budding (the cells indicated by red arrows), and metastatic lymph nodes in the same patient (B). Representative stained images of H2AX (D) and E-cadherin (F) in central tumors and metastatic lymph nodes in the same patient. Scale bar = 300 μm.

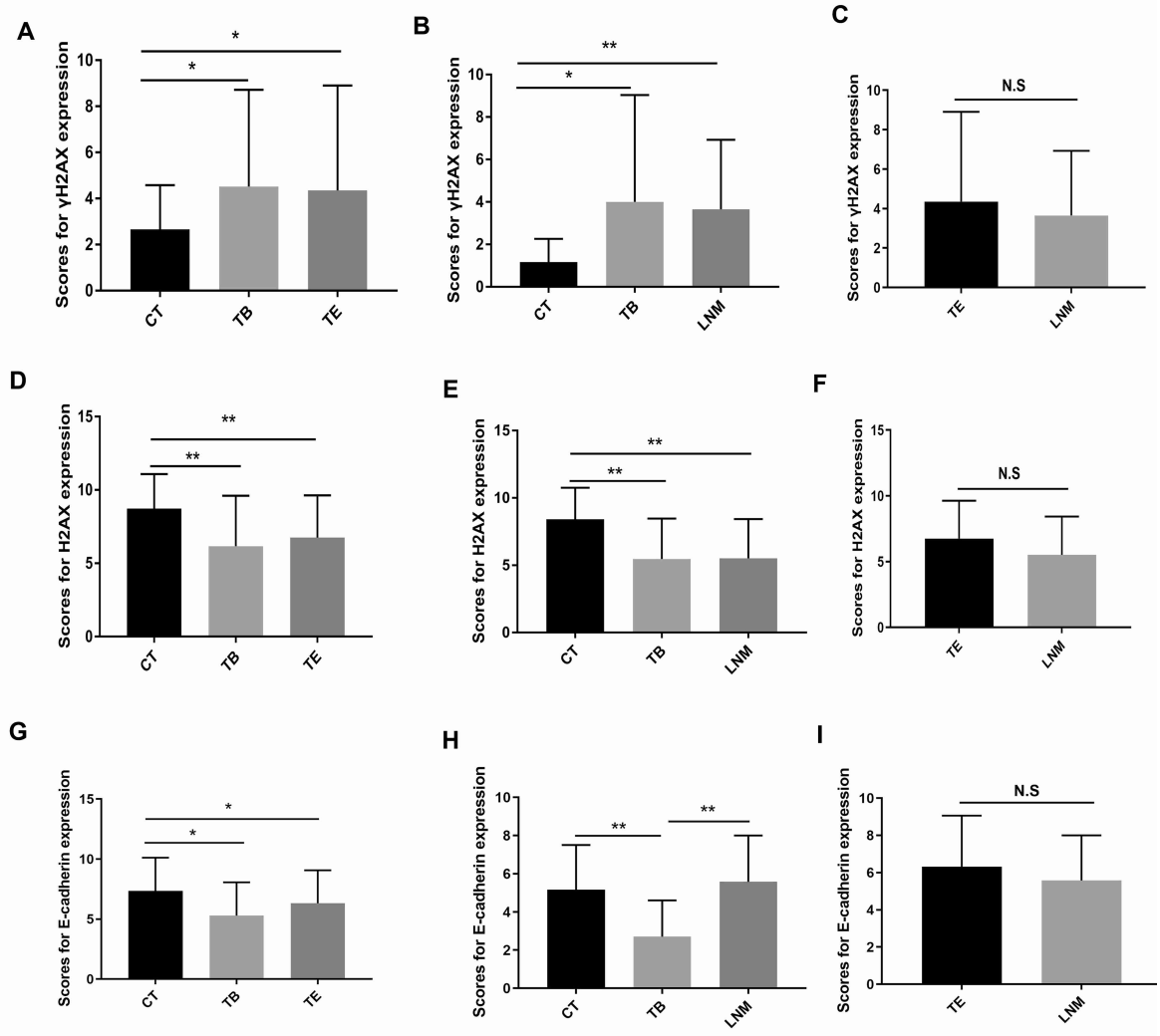

FIGURE 4. Changes of γH2AX, H2AX and E-cadherin expression in central tumors, tumor budding, tumor emboli and metastatic lymph nodes. Comparison of γH2AX (A), H2AX (D) and E-cadherin (G) expression in central tumors, tumor budding, and tumor emboli in the same patients (N = 18). Comparison between the expression levels of γH2AX (B), H2AX (E), and E-cadherin (H) in central tumors, tumor budding and metastatic lymph nodes in the same patients (N = 16). Comparison between the expression levels of γH2AX (C), H2AX (F), and E-cadherin (I) in tumor emboli and metastatic lymph nodes (N = 18 and N = 16, respectively). CT: central tumors; TB: tumor budding; TE: tumor emboli; LNM: Metastatic lymph nodes. *, $p < 0.05$; **, $p < 0.01$.

3.4 H2AX expression was negatively correlated with γH2AX expression and positively correlated with E-cadherin expression in CIN, central tumors, tumor budding and lymph node metastasis.

To investigate the relationship between γH2AX, H2AX and E-cadherin, we further analyzed the correlations of their expression levels in CIN, central tumors, tumor budding and lymph node metastasis. Analysis revealed a significant negative correlation between γH2AX expression and H2AX expression in cervical lesions ($r = -0.243$, $p < 0.001$; Fig. 5A). Furthermore, H2AX expression exhibited a positive correlation with E-cadherin expression ($r = 0.491$, $p < 0.001$; Fig. 5B). No significant correlation was detected between γH2AX and E-cadherin expression ($r = -0.039$, $p > 0.05$; Fig. 5C).

3.5 Higher expression levels of γH2AX in tumor budding was associated with the presence of tumor emboli and International Federation of Gynecology and Obstetrics (FIGO) staging scores

Finally, we investigated the correlation between the expression of γH2AX and H2AX in central tumors or tumor budding and the clinicopathological characteristics of patients. Analysis showed that higher expression levels of γH2AX in tumor budding were associated with tumor emboli, pausimenia and FIGO staging scores (all $p < 0.05$, Table 1). However, there was no significant correlation between the expression levels of H2AX and clinicopathological features, either in central tumors or tumor budding (all $p > 0.05$, Table 1).

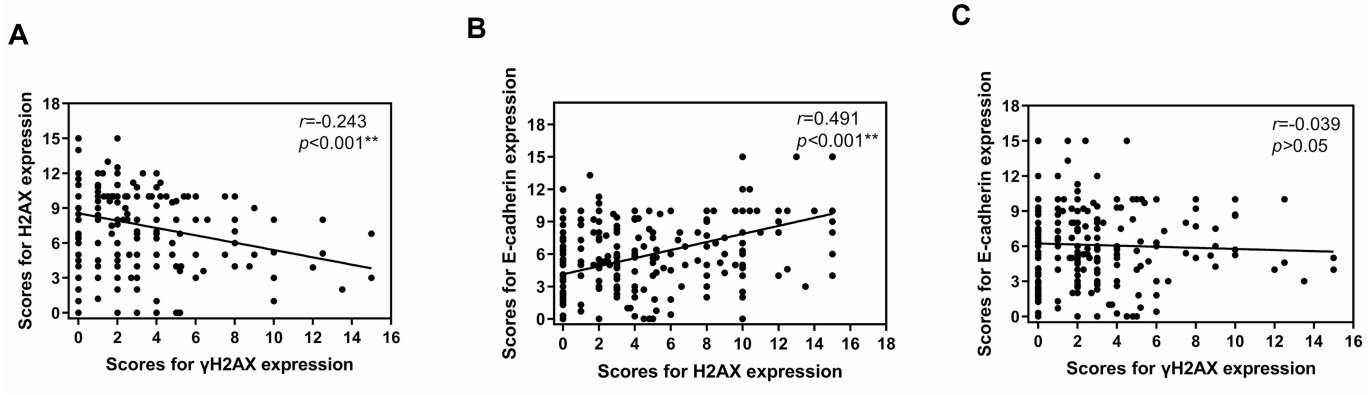

FIGURE 5. Correlations of γH2AX, H2AX and E-cadherin expression in central tumors, tumor budding, tumor emboli and metastatic lymph nodes. (A) Correlation between γH2AX and H2AX. (B) Correlation between H2AX and E-cadherin. (C) Correlation between γH2AX and E-cadherin. **, $p < 0.01$.

4. Discussion

Tumor markers can be defined as molecular products expressed by tumor tissues by immunohistochemistry or metabolized and secreted by tumors and biochemically characterized in body fluids such as blood and urine. These markers serve as indicators for tumor staging and grading, as well as for monitoring treatment response and predicting recurrence, progression, metastasis and patient survival. Currently, there are numerous promising biomarkers available for gynecological tumors. For instance, in endometrial cancer, *astrocyte elevated gene-1* (*AEG-1*), microRNAs (miRNAs), and the combination of human epididymis protein 4 (HE4) with traditional markers (CA125, CA724 and CA19-9) are considered to show significant potential. Ovarian cancer presents cancer-testis antigens (CTAs) and circulating tumor DNA (ctDNA) as relevant markers, while cervical cancer involves miRNAs, P16 and Ki67 [21]. However, none of these biomarkers are commonly utilized in clinical applications. γH2AX, considered a marker of genomic instability, plays a role in the development of biliary tract carcinogenesis and is associated with pancreaticobiliary maljunction [22], as well as bladder carcinogenesis [23]. The expression of γH2AX is significantly elevated in precancerous lesions of the liver [5], ovaries [6], and gastrointestinal stromal tumors (GISTs) [7], when compared with corresponding benign tumors or normal cells and tissues. Breast cancer is associated with high expression levels of γH2AX [24] and a positive correlation with poor histopathological parameters [25]. Furthermore, γH2AX serves as a sensitive and specific marker for the early detection of hepatocellular carcinoma [26] and bladder cancer [27]. Collectively, these findings suggest that γH2AX shows promise as a marker for tumorigenesis and the progression of disease. Our analysis showed that the expression of γH2AX increased significantly with the progression of CIN to cervical cancer, thus suggesting that γH2AX may also serve as a potentially useful marker for the development of cervical cancer. This observation is also consistent with our previous study [10] and a study reported by Brustmann *et al*. [28].

Our analysis revealed that γH2AX expression in tumor budding was significantly higher than in central tumors and that the higher expression levels of γH2AX in tumor budding was significantly associated with the presence of tumor emboli, thus indicating that γH2AX may promote the invasion of tumor cells. In this study, we found no significant difference in γH2AX expression when compared between tumor budding, tumor emboli and metastatic lymph nodes, although its expression was significantly higher in these regions when compared to central tumors. These results suggested that a marked increase in γH2AX expression is necessary for the transition of primary tumor cells (with or without low metastatic potential) to metastatic cells (with high metastatic potential). Furthermore, the consistent pattern of γH2AX overexpression during metastasis suggests that its pro-metastatic role can extend from tumor bud formation to lymph node metastasis in cervical cancer. This was also confirmed by our finding that high expression levels of γH2AX in tumor budding were correlated with FIGO staging. We analyzed the correlation between γH2AX and E-cadherin but found no significant correlation. This suggests that γH2AX contributes to carcinogenesis, invasion and metastasis via mechanisms that are independent of or not directly related to EMT. Interestingly, we found that the expression of γH2AX was higher in tumor budding from premenopausal patients with cervical cancer. In North America, the postmenopausal population is considered a high-risk group for cervical cancer. This is primarily due to either the discontinuation of cervical cancer screening in women over the age of 65 years or inadequate screening [29] which is not influenced by hormonal changes. The presence of increased levels of estrogen can modify the validated microenvironment of the cervix, potentially contributing to cervical carcinogenesis and the progression of cervical cancer [30]. Based on this rationale, we hypothesized that the elevated expression of γH2AX in premenopausal patients may be associated with these factors.

H2AX, as a precursor protein of γH2AX, can maintain the proliferative function of endothelial cells under hypoxic conditions, thereby promoting neovascularization [31]; this can provoke the metastasis of invasive breast cancer cells [32]. Furthermore, high expression levels of γH2AX are associated with increased DNA repair, cell proliferation, metastasis and the poor survival of patients with breast cancer [33]. How-

TABLE 1. The relationship between the expression levels of γH2AX and H2AX in central tumors and tumor budding, and the clinicopathological characteristics of patients with SCC.

Characteristic	N	γH2AX (H score, Mean ± SD)				H2AX (H score, Mean ± SD)			
		CT	*p*-value	TB	*p*-value	CT	*p*-value	TB	*p*-value
Age (yr)									
<50	39	2.4 ± 2.3	0.688	3.5 ± 4.1	0.190	8.4 ± 2.9	0.314	5.5 ± 4.0	0.596
≥50	39	2.2 ± 2.2		2.4 ± 3.1		7.7 ± 2.9		5.0 ± 3.5	
Pathological type									
Keratin type	40	2.4 ± 2.3	0.552	3.0 ± 3.5	0.970	8.4 ± 2.4	0.289	5.4 ± 3.6	0.679
Non-keratinizing type	38	2.1 ± 2.2		3.0 ± 3.8		7.7 ± 3.4		5.1 ± 3.9	
Infiltrating depth									
<1/2	13	2.2 ± 2.3	0.932	3.2 ± 4.6	0.809	7.0 ± 4.2	0.111	4.7 ± 4.1	0.568
≥1/2	65	2.3 ± 2.4		2.9 ± 3.5		8.4 ± 2.4		5.3 ± 3.7	
FIGO stage									
I	37	1.7 ± 1.9		1.7 ± 2.5		8.2 ± 3.5		5.3 ± 4.1	
IIa	19	2.3 ± 2.1	0.062	3.0 ± 3.1	0.012*	8.2 ± 2.5	0.911	5.1 ± 3.4	0.980
IIb–III	22	3.2 ± 2.6		5.0 ± 4.8		7.8 ± 2.3		5.3 ± 3.5	
Tumor differentiation									
Well and medium	64	2.3 ± 2.3	0.626	3.0 ± 3.8	0.740	8.3 ± 2.7	0.124	5.1 ± 3.6	0.593
Poor	14	2.0 ± 2.0		2.7 ± 2.9		7.0 ± 3.6		5.8 ± 4.6	
Tumor emboli									
Yes	27	2.8 ± 2.5	0.124	4.5 ± 4.6	0.018*	7.8 ± 3.1	0.636	5.1 ± 3.6	0.774
No	51	2.0 ± 2.0		2.2 ± 2.9		8.2 ± 2.8		5.3 ± 3.8	
Tumor budding									
<9	54	2.4 ± 2.1	0.640	3.0 ± 3.5	0.981	7.7 ± 3.2	0.063	4.8 ± 3.7	0.138
≥10	24	2.1 ± 2.5		3.0 ± 3.0		8.6 ± 2.0		6.2 ± 3.9	
Perineural invasion									
Yes	9	2.7 ± 3.3	0.517	3.6 ± 4.0	0.606	8.2 ± 2.5	0.865	6.1 ± 3.1	0.491
No	69	2.2 ± 2.1		2.9 ± 3.6		8.1 ± 3.0		5.1 ± 3.8	
Lymph node metastasis									
Yes	18	3.1 ± 3.0	0.163	4.1 ± 4.7	0.144	8.5 ± 2.2	0.503	5.8 ± 3.1	0.443
No	60	2.0 ± 1.9		2.6 ± 3.2		8.0 ± 3.1		5.1 ± 3.9	
Pausimenia									
Yes	25	2.0 ± 2.2	0.439	2.0 ± 2.5	0.049*	7.7 ± 3.2	0.396	5.1 ± 3.5	0.868
No	53	2.4 ± 2.2		3.4 ± 4.0		8.3 ± 2.8		5.3 ± 3.9	

*SCC: squamous cell carcinoma; CT: central tumor; TB: tumor budding; FIGO: International Federation of Gynecology and Obstetrics; SD: Standard Deviation. *, $p < 0.05$.*

ever, other studies have revealed that H2AX functions as a tumor suppressor. The depletion of H2AX activates the EMT program of cancer cells, which plays an important role in tumor development, progression and metastasis [12, 13, 34]. Mice with one or both copies of H2AX removed from a p53 mutant background developed unstable genomes, including translocations and tumors [35]. These findings suggest that the role of H2AX is presently controversial. In this study, the expression levels of H2AX were significantly lower in SCC than that in CIN 2/3, and significantly lower in CIN 2/3 than that in CIN 1, thus suggesting that H2AX may play a tumor suppressive role in the progression of CIN to cervical cancer. We further observed that H2AX expression in tumor budding was significantly lower than that in central tumors, thus suggesting that reduced levels of H2AX expression are associated with the invasion of cervical cancer. We also detected a positive correlation between H2AX and E-cadherin expression, thus indicating that H2AX may contribute to the invasion of cervical cancer by participating in the EMT program of cervical cancer. In addition, we observed that in some cases, tumor cells at the margins of cancer nests were spindle-shaped, and that the levels of H2AX expression in these cells was significantly reduced. It is well known that tumor cells tend to exhibit a morphology similar to spindle-shaped mesenchymal cells when undergoing EMT; therefore, we hypothesized that H2AX expression was reduced in tumor cells undergoing EMT; this is consistent with the findings described above. Studies have reported that primary tumors and metastatic nodules express similar levels of key markers of the EMT program [34, 36]. Our findings demonstrated that the expression levels of H2AX in tumor thrombi or metastatic lymph nodes were significantly lower than those in central tumors, although there was no significant difference in H2AX expression between tumor thrombi, metastatic lymph nodes, and tumor budding. These results may be related to the involvement of H2AX in the development of EMT in cervical cancer.

During DNA damage, γH2AX is generated after the phosphorylation of Ser139 in H2AX. In this study, we found that γH2AX expression gradually increased, while H2AX expression gradually decreased with the severity of cervical lesions. These results prompted us to evaluate the correlation between H2AX and γH2AX. We detected a negative correlation, thus suggesting that the reduction in H2AX expression may be related to DSB. In a previous study, we found that HPV infection was associated with DSB [37]. Other studies showed that the expression of HPV proteins increased the levels of reactive oxygen species (ROS), thus leading to DNA damage [38–40]. Chronic oxidative stress due to increased levels of ROS can increase the E3 ubiquitin ligase *RNF168*-mediated ubiquitination of H2AX, ultimately leading to the degradation of H2AX by the proteasome [41]. Therefore, we hypothesized that the increased expression of γH2AX and the reduced expression of H2AX, along with their negative correlation, might be caused by the increased levels of ROS due to HPV infection; however, this hypothesis has yet to be confirmed.

cadherin, a calcium-dependent cell-cell adhesion molecule, plays a pivotal role in EMT. The absence of E-cadherin not only disrupts intercellular connections but also facilitates intracellular signaling cascades, ultimately promoting tumor invasion and metastasis [42]. In the context of cervical cancer screening, the combined assessment of the DNA methylation of E-cadherin and the *p15* gene exhibits a superior sensitivity and specificity of 80% and 90%, respectively, thus surpassing that of the traditional Papanicolaou test (PAP smear) [43]. Furthermore, E-cadherin is important in the diagnosis of adenocarcinoma of the uterine cervix in Silva-type staging [44]. While magnetic resonance imaging (MRI) currently serves a pivotal role in identifying the anatomical origin of endocervical or endometrial cancer [45], E-cadherin may serve as a potential new marker, alongside MRI, to distinguish the tissue origin of uterine cervical masses. In this study, we found that the expression of E-cadherin decreased with increased disease severity in both CIN and SCC. Furthermore, E-cadherin expression was significantly lower in tumor budding than in central tumors, thus suggesting that E-cadherin deficiency plays an important role in the progression and invasion of cervical cancer. We also observed that the expression levels of E-cadherin in metastatic lymph nodes were higher than those in tumor emboli and tumor budding, but not significantly different from those in central tumors, thus indicating that tumor cells metastasized to lymph nodes regained epithelial cell markers. This finding is consistent with the characteristics of tumor cells undergoing MET, thus suggesting that E-cadherin also plays a role in the metastatic colonization of tumors. These findings align with previous research conducted by Padmanaban *et al*. [46]. Due to our primary focus, a detailed discussion about the role of E-cadherin in cervical cancer is beyond the scope of this paper.

This study has some limitation that need to be considered. Due to the small sample size for some specimens, only a certain level of statistical analysis could be performed. For example, the numbers of specimens with tumor emboli and lymph node metastasis were insufficient. In the future, we will continue to expand the number of cases to improve the representativeness of the samples and the accuracy of the data analysis.

5. Conclusions

In summary, our study clarifies the distinct contributions of γH2AX and H2AX to cervical cancer progression. While both markers are implicated in carcinogenesis and metastasis, their functional divergence is striking: H2AX drives invasion and metastasis through EMT activation, whereas γH2AX, despite its association with DNA damage response, shows no direct involvement in EMT. This functional divergence positions H2AX as a promising therapeutic target for metastasis suppression, while γH2AX's lack of EMT involvement suggests its role may be confined to earlier carcinogenic stages. However, the relatively small cohort, particularly the limited cases with tumor emboli and lymph node metastasis, constrained the statistical power of subgroup analyses. Future studies will prioritize expanding the cohort to enhance sample representativeness and data reliability.

AVAILABILITY OF DATA AND MATERIALS

The data from this study is available upon reasonable request to the corresponding author.

AUTHOR CONTRIBUTIONS

JZ and ZG—conceived and designed this study; edited the manuscript. LS, XQL and YHS—performed immunohistochemical staining. LS—drafted the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This trial was approved by the ethics committee of Northwest Minzu University (approval number: XBMZ-YX-2020026). The trial was registered in the Chinese Clinical Trial Registry (http://www.chictr.org.cn; registration no. ChiCTR-TRC-1800016405; principal investigator: Zhong Guo; date of registration: 31 May 2018). Written informed consent was obtained from each patient participating in this study.

ACKNOWLEDGMENT

We thank Englishgo (https://englishgo.net/) for editing this manuscript.

FUNDING

This study was supported by the Fundamental Research Funds for the Central Universities (nos. 31920190210 and 31920220012), the Gansu Province Natural Science Foundation (no. 20YF8FA046), and the National Natural Science Foundation of China (no. 82160509).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Lina Shang, Xiaoqin Li, Yihang Song, Jin Zhao, Zhong Guo. The role of γH2AX and H2AX in cervical carcinogenesis, invasion, and metastasis, and their relationship with the expression of E-cadherin. European Journal of Gynaecological Oncology. 2025; 46(3): 16-26. doi: 10.22514/ejgo.2025.032.