EJGO European Journal of Gynaecological Oncology

Expressions of H2AX in cervical squamous carcinoma and their clinical significances

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Summary

Objective: To evaluate the expression and clinical significance of H2AX in cervical squamous carcinoma. *Materials and Methods.* The expression of H2AX in the cervical squamous carcinoma of 37 patients and in the normal cervical tissue of 15 patients was detected by immuneohistochemical method. Chi-square, correlation analysis, and Kaplan-Meier were utilized to analyze the data. *Results:* The positive expression rate of H2AX in cervical squamous carcinoma was higher than that in normal cervical tissues ($p = 0.016$) . The expression of H2AX was negatively correlated with FIGO staging, vaginal invasion, and parametrial infiltration ($p < 0.05$). Survival analysis showed the expression intensity of H2AX had no significant effect on the prognosis. *Conclusion:* H2AX was an anticancer protein for cervical squamous carcinoma.

Key words: Cervical squamous carcinoma; H2AX; Phosphorylate; Immunohistochemistry.

Introduction

H2AX is a variant of H2A, which was firstly identified as isomorphisms of H2A in 1980 [1]. The unique feature of H2AX is a conservative serine residue in C-terminus. The serine residue is phosphorylated rapidly to form γ-H2AX when DNA is damaged and double-strand break (DSB) occurs. γ-H2Ax can collect the repair factors and help the repair of DNA. γ-H2AX is timely dephosphorylated after the repair of DNA is completed. It has been found that γ-H2Ax has a relation with genetic transcription and regulation, DNA repair, cell cycle control, and regulation of cell apoptosis [2-4]. The present research utilized immunohistochemistry to detect the expression of H2AX in cervical squamous carcinoma. The expression in cervical squamous carcinoma and normal cervical tissue was compared. Found The relationship between the expression of H2AX and histological grade, FIGO staging, lymphatic metastasis, parametrial infiltration, vaginal invasion, vascular metastasis, myometrial invasion and age was also assessed, including the effect of H2AX on prognosis.

Materials and Methods

Material

Thirty-seven cases of cervical squamous carcinoma were chosen randomly that had radical hysterectomy and pelvic lymph node dissection in the present hospital during 2006 to 2007 (Table 1). The average age of the patients was 46 ± 9.803 years. During follow-up through 2014, 16 patients died. Control group consisted of 15 cases with normal cervical tissue.

Immunohistochemistry method

Formalin-fixed paraffin-embedded slides were used to identify representative sections of tumor and normal tissue. The tissue was stained using anti-histone H2AX (phospho S139) at a concentration of 1:50. Antigen was retrieved by EDTA (pH 9.0) microwave antigen retrieval. PBS was used instead of primary antibody in the negative control. Breast cancer tissue was used in positive control.

Method of observation and judgment of results

Histological images were captured with a microscope with an objective magnification of ×40. The proportion of positive tumor cells was scored as: 0 = less than 10%; 1+ = 10%–30%; 2+ = 31%–50%; 3+ = 51-80%; and 4+ > 80%. The intensity was arbitrarily scored as 0 = weak (no color or light blue), 1 = moderate (light yellow), 2 = strong (yellow brown), and 3 = very strong (brown) (Table 2). The overall score was calculated by multiplying the two scores obtained from each sample. A score of ≥ 4 was defined as high H2AX expression and a score of < 4 was defined low H2AX expression.

Statistical method

Data was analyzed by SPSS16.0. The relationships between the expression of H2AX and clinical pathological features were detected by chi-square analysis and correlation analysis. Prognosis was determined by Kaplan-Meier analysis.

Results

The expression of H2AX in cervical squamous carcinoma was obviously higher than that in normal cervical tissues ($p = 0.016$) (Table 3) (Figure 1-6). The expression in cervical squamous carcinoma had a negative correlation

Revised manuscript accepted for publication September 28, 2015

Eur. J. Gynaecol. Oncol. - ISSN: 0392-2936
XXXVIII, n. 1, 2017
doi: 10.12892/ejgo3304.2017

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Table 1. — *Clinical pathological features.*

Cases		Cases	
Stage		Vascular metastasis	
I	26	no	15
II	11	yes	22
Lymphatic metastasis		Myometrial invasion	
no	26	≤1/2	9
yes	12	>1/2	28
Parametrial infiltration		Age	
no	33	< 50	25
yes	4	≥ 50	12
Vaginal invasion		Histological grade	
no	28	low	28
yes	9	middle	7
		high	2

Table 2. — *Evaluation criterion.*

Intensity	No staining	Yellow	Yellow brown	Brown	
Score	0	1	2	3	
Proportion	≤10%	11%~30%	31%–50%	5l%–80%	≥81%
Score	0	1	2	3	4

Table 3. — *Result of chi-square analysis.*

H2A expression	Negative	Positive	*p*
Cervical squamous carcinoma	16	21	0.016
Normal cervical tissue	12	3	

Table 4. — *Result of chi-square analysis.*

H2AX expression		Negative	Positive	*p*
FIGO Stage	I	8	18	0.023
	II	8	3	
Lymphatic metastasis	No	9	17	0.103
	Yes	8	4	
Parametrial infiltration	No	12	21	0.028
	Yes	4	0	
Vaginal invasion	No	9	19	0.022
	Yes	7	2	
Vascular metastasis	No	6	9	0.505
	Yes	10	12	
Myometrial invasion	≤ 1/2	6	3	0.107
	> 1/2	10	18	
Histological grade	Low	11	17	0.138
	Middle	5	2	
	High	0	2	
Age	< 50	10	15	0.411
	≥ 50	6	6	

Table 5. — *Result of correlation analysis.*

Clinical pathological features	Correlation index	*p*
FIGO Stage	-0.387	0.009
Parametrial infiltration	-0.399	0.007
Vaginal invasion	-0.395	0.008

with FIGO Stage, parametrial infltration, and vaginal invasion (Tables 4 and 5). The survival analysis showed that the expression of H2AX had no obvious effect on the prognosis of cervical squamous carcinoma (Figure 7).

Discussion

H2AX is the most sensitive factor which responds to DNA damage. DSBS, the most serious DNA damage, can lead to the phosphorylation of H2AX to form γH2AX. Many researches have shown that γH2AX is an ideal protein marker of DNA marker [5, 6]. γH2AX can recognize and repair DNA damage, interact with p53, active cell cycle checkpoint, block cell cycle and, correctly ensure the hereditary information. When DNA repair cannot be completed, γH2AX will block cell cycle and induce cell apoptosis. It was reported that imatinib mesylate could induce gastrointestinal tumor cell line apoptosis through upregulation of H2AX [7].

H2AX is located in chromosome 11(11q23). The deficiency and translocation of this region occurs in many human tumors, such as hematological malignancy [8] and solid tumor. Parikh *et al.* [9] found that the deficiency of 11q23 in head and neck neoplasms. Bassing *et al.* found that mice which had H2AX knocked out were sensitive to ionizing radiation and tumor [10]. Without H2AX, the DNA cannot be repaired and the stability of gene is destroyed. Olive [11] found that γH2AX was in relation with DNA damage, tumor occurrence, and development. The main reason was the decrease of gene stability. γH2AX is an important cancer suppressor gene which had important effect on the stability of heredity [12]. It has been found that the expression of γH2AX obviously increases in cancer and precancerosis, accompanying with activations of other DNA damage response (DDR), for example cHK2, p53, and 53BP1.These researches all provided the evidences that H2AX could suppress the tumor in humans [13].

Radiotherapy and chemotherapy are the main methods to treat tumor, especially advanced and recurrent ones. Many chemotherapeutics kill tumor by inducing DSBs. Kunos *et al.* [14] reported that salinomycin could increase DNA damage, which could promote tumor sensitivity to adriamycin and etoposide and decrease P21 level. After combined treatment of salinomycin, adriamycin, and etoposide, the level of γH2AX, p53BP1, and pChk1 increased. Ivashkevich *et al.* [15] found that the level of γH2AX increased in peripheral blood lymphocyte after

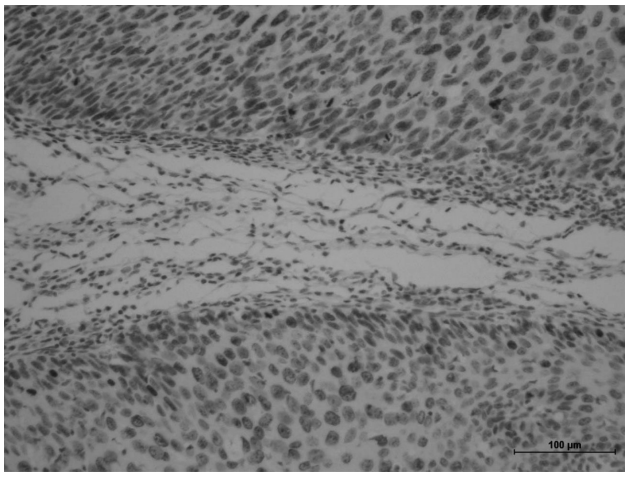

Figure 1. — Positive expression of H2AX in cervical squamous carcinoma.

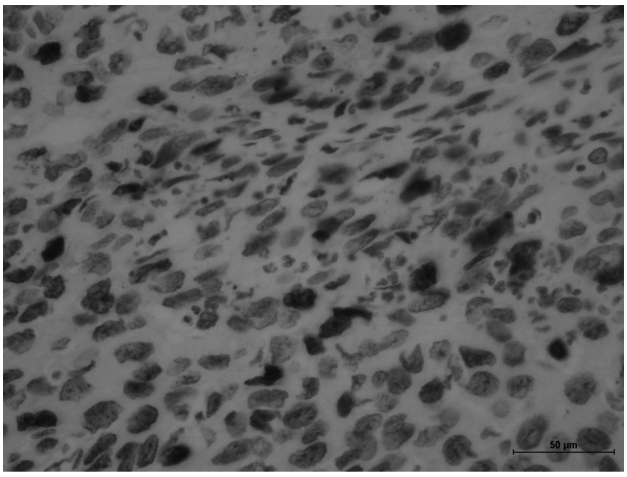

Figure 2. — Positive expression of H2AX in cervical squamous carcinoma.

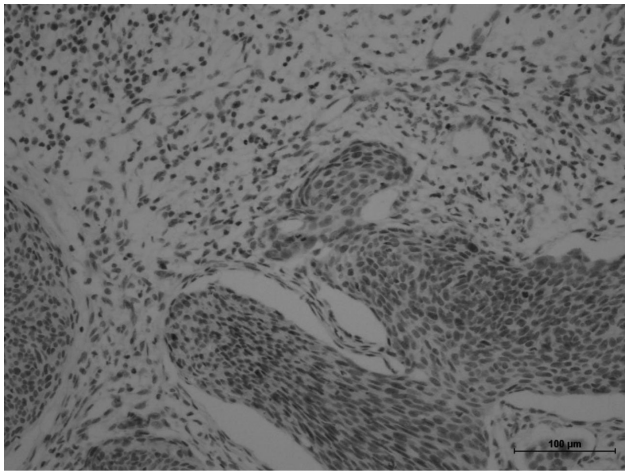

Figure 3. — Negative expression of H2AX in cervical squamous carcinoma.

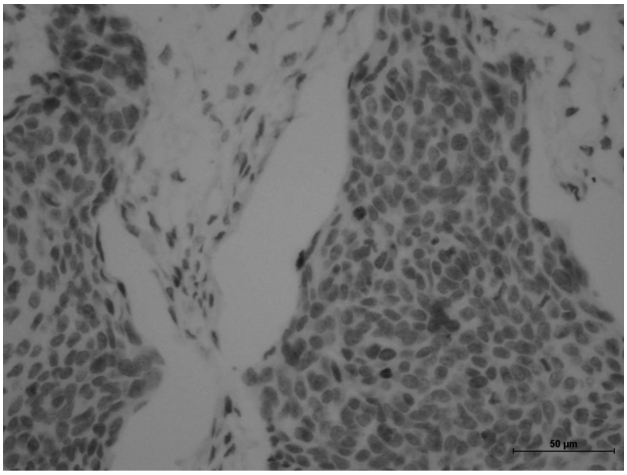

Figure 4. — Negative expression of H2AX in cervical squamous carcinoma.

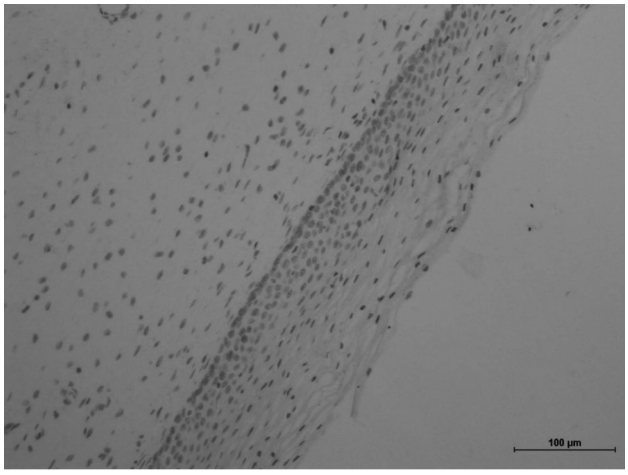

Figure 5. — Negative expression of H2AX in normal cervical tissue.

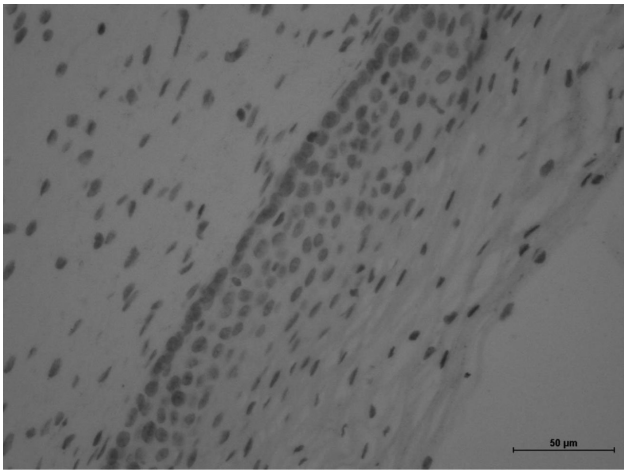

Figure 6. — Negative expression of H2AX in normal cervical tissue.

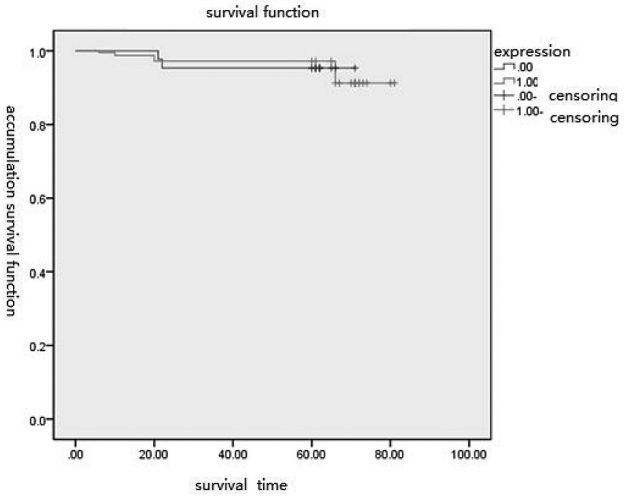

Figure 1. — Survival curve.

using DNA methyltransferase inhibitor to treat myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML). Zolner *et al.* [16] found that ionizing radiation could cause mutation of human phosphorylation site, which stopped the H2AX phosphorylation, lowered the quality of DNA repairm and finally destroying the tumor.

The present research utilized immunohistochemistry method to detect the expression of H2AX in cervical squamous carcinoma. The present authors found that the expression of H2AX in cervical squamous carcinoma was obvious higher than that in normal cervical tissue. The expression of H2AX was higher in early-stage cervical squamous carcinoma than in late stage, which provides evidence to the theory that H2AX is an anticancer protein. The present authors hope that this research could assist early diagnosis and treatment of cervical squamous carcinoma; it could also be used to detect the curative effect of chemotherapy and radiotherapy.

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