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Quantitative Differences in Levels of Immunohistochemical Biomarkers between Squamous Cell Carcinoma and Adenocarcinoma of the Uterine Cervix: Implications for Treatment Outcomes after Chemoradiotherapy

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Abstract

Introduction: This study compared the quantitative differences in immunohistochemical markers between uterine cervical squamous cell carcinoma (SCC) and adenocarcinoma (AC) and assessed the impact of these biomarkers on outcomes in patients treated with chemoradiotherapy (CRT).

Method: This retrospective study included 118 patients (SCC in 76, AC in 42) who received definitive CRT. According to the International Federation of Gynecology and Obstetrics staging system, 14, 34, and 70 patients were classified as having stage IB3, II, and III disease, respectively. Baseline immunohistochemical biomarkers, including hypoxia, cell proliferation, cell adhesion, immunogenicity, inflammatory, and evasion of apoptosis biomarkers, *were analyzed using tissue microarrays from biopsy specimens. The* Mann-Whitney U test was carried out for quantitative analysis between SCC and AC. *Cox regression* analysis was used to examine the effects of the biomarkers and clinical parameters on treatment outcomes.

Results: Using the H-scores of the biomarkers for SCC as a reference, increased expression of E-cadherin, calretinin, *CAIX, and c-Myc* and decreased levels of HIF-1α, VEGF, tumor necrosis factor-α, galectin-9, chemokine ligand 5, Bax, EGFR, and insulin-like growth factor 1 receptor were found in the patients with AC. A high E-cadherin (P = 0.002) and low Bax (P = 0.001) H-score were associated with inferior pelvic relapse-free survival.

Conclusion: Cervical SCC exhibited strong expression of baseline immunohistochemical inflammatory, hypoxic, and angiogenesis biomarkers whereas the intensity of cell adhesion markers was more distinct in cervical AC. A high E-cadherin and a low Bax H-score were associated with a high rate of local relapse.

Introduction

Uterine cervical cancer is both the fourth most common cause of cancer and the fourth most common cause of death from cancer in women worldwide[1]. Squamous cell carcinoma (SCC) has the highest incidence, but the incidence of adenocarcinoma (AC) has increased in recent decades, and constitutes approximately 10–20% of all cervical carcinomas[2–4]. Some studies indicated that cervical AC and SCC behave differently epidemiologically[3–7], and have different genomic expressions [8, 9]. In addition, they have diverse prognostic factors and patterns of failure after similar treatments[5, 7] [10–13]. As advances in molecular profiling have allowed for the identification of biomarkers of many biological characteristics in tumor cells, biomarkers in standard treatment are of interest for their potential role in the design of personalized therapeutic strategies targeting individual tumors, rather than therapy based on histological types alone.

Given that chemoradiotherapy (CRT) has been the standard of care for patients with locally advanced cervical cancer worldwide, radioresistance or treatment failure is a clinically relevant problem. Patients with cervical AC primarily treated with radiotherapy have inferior outcomes compared with those with SCC[5, 12–14]. In cervical cancer, several biomarkers for radiotherapy-based treatment have been validated by patient survival or recurrence data[15, 16]. These biomarkers fall into categories according to biological function including hypoxia, cell proliferation, cell adhesion, immunogenicity, and evasion of apoptosis[15]. To optimize the treatment outcomes for patients with advanced disease, there is a great need to understand the differences in the expression of the aforementioned biomarkers between cervical SCC and AC, particularly for CRT-based prognostic factors. Hence, this study was conducted to investigate the quantitative differences in immunohistochemical (IHC) biomarkers between the two pathologies. Thus, the impact of these IHC markers on CRT-based treatment beyond the histological types could be identified.

Materials And Methods

Study population

For this study, incisional biopsy specimens from patients newly diagnosed with stage IB3 to III uterine cervical SCC and AC between July 2009 and December 2015 were analyzed. Because of missing tumor tissue in some specimens, this retrospective study included a cohort consisting of 76 SCC and 42 AC patients. According to institutional protocol, all patients had undergone F-18 fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) for initial staging. In addition, all had received allocated external-beam radiotherapy and intracavitary brachytherapy. Concurrent chemotherapy consisted of weekly administration of 40 mg/m^2 cisplatin. The patients were staged in accordance with the International Federation of Gynecology and Obstetrics[17]. Accordingly, 14, 34, and 70 patients were classified as having stage IB3, II, and III disease, respectively. The median age of our patients was 55 years. Because FDG-PET/CT has high sensitivity and specificity in detecting the nodal status in cervical cancer, PET/CT was used for the diagnosis of pelvic or paraaortic lymph node metastasis. We excluded patients who were diagnosed as having a histological type of adenosquamous

carcinoma. This study was approved by the local institutional review board [CMUH-107-REC3-163]. Patient characteristics are listed in Table 1.

Table 1
Patient characteristics

Variables	Squamous cell carcinoma (n = 76)	Adenocarcinoma (n = 42)
Age (year)	median 56 (range, 24 ~ 77)	median 55 (range, 33 ~ 77)
FIGO stage		
IB3	6 (8%)	8 (19%)
IIA-IIB	21 (28%)	13 (31%)
IIIA-IIIC2	49 (64%)	21 (50%)
Maximum tumor dimension (cm)	mean 5.1 ± 0.9 (range, 3.5 ~ 8.3)	mean 5.7 ± 1.1 (range, 3.9 ~ 8.6)
Pelvic lymph node metastasis		
negative	34 (45%)	24 (57%)
positive	42 (55%)	18 (43%)
Paraaortic lymph node metastasis		
negative	65 (86%)	38 (90%)
positive	11 (14%)	4 (10%)
Pretreatment hemoglobulin (g/dL)	mean 11.8 ± 1.8 (range, 7.6 ~ 15.3)	mean 10.3 ± 3.0 (range, 3.5 ~ 14.3)
External beam radiotherapy (cGy)		
whole pelvis (Gy)	median 45 (range, 45 ~ 54)	median 45 (range, 45 ~ 54)
bilateral parametrium boost with central shielding (Gy)	median 54 (range, 50.4 ~ 57.6)	median 54 (range, 50.4 ~ 57.6)
pelvic lymph node boost (Gy)	median 64 (range, 60 ~ 66)	median 64 (range, 60 ~ 66)
Brachytherapy		
2 dimensional brachytherapy (6 Gy to point A per session for 4–5 courses)	51	14
Cumulative EQD2 to point A (Gy_{10})	mean 77.4 ± 6.8	mean 84.3 ± 7.9
3 dimensional brachytherapy (HR-CTV > 6.5 Gy per session for 4 to 5 courses)	25	28
Cumulative EQD2 of D90 of HR-CTV (Gy_{10})	mean 87.2 ± 8.3	mean 88.1 ± 10.3

Abbreviations: FIGO = International Federation of Gynecology and Obstetrics; EQD2 = equivalent dose in 2 Gy; and HR-CTV = high-risk clinical target volume ;

Immunohistochemistry

As reported in our previous study[18], IHC biomarkers, namely endogenous hypoxic (Glut1, CAIX, and HIF-1α), angiogenesis or metastasis (VEGF), cell proliferation [EGFR, c-Myc, insulin-like growth factor 1 receptor IGF-1R)], cell to cell adhesion (E-cadherin, Vimentin, calretinin), evasion to apoptosis [B-cell lymphoma 2 (Bcl-2), Bax, myeloid cell leukemia 1 (Mcl-1)], and immunogenic or inflammatory biomarkers [programmed cell death protein ligand 1 (PD-L1),} tumor necrosis factor-α (TNF-α), galectin-9, and chemokine ligand 5 (CCL5)] were analyzed using tissue microarrays from incisional biopsy specimens before treatment. Each tumor was represented by one tissue core on a tissue microarray. Paraffin Sect. 4-μm-thick were deparaffinized and microwaved according to standard procedures before being processed for IHC staining.

The staining slides were scored by 2 pathologists blinded to the clinical outcome. Except for PD-L1, IHC results of the aforementioned biomarkers were scored by a semiquantitative approach used to assign an H-score to tumor samples[19]. The H-score takes into consideration the staining intensity in conjunction with the percentage of cells staining positively. Staining intensity was graded as 0, 1, 2, and 3 corresponding to negative, mild, moderate, and strong, respectively. The percentage of positive tumor cells was estimated by the observers. The total number of neoplastic cells in the field and the number of neoplastic cells stained at each intensity were counted. The following formula was applied: H-score = [% of cells stained at intensity category 1 (neoplastic cells with mild staining) x 1] + [% of cells stained at intensity category 2 (neoplastic cells with moderate staining) x 2] + [% of cells stained at intensity category 3 (neoplastic cells with strong staining) x 3]. Accordingly, the H-scores, ranging from 0 to 300, were calculated with 300 equal to 100% of tumor cells stained strongly (3 +).

Tumor PD-L1 biomarker was evaluated through IHC staining using the DAKO clone 22C3 pharmDx kit (DAKO, Carpinteria, CA,USA). PD-L1 expression was scored according to the combined positive score (CPS), which is the number of PD-L1 stained cells (tumor cells, lymphocytes, macrophages) at any intensity divided by the total number of viable tumor cells, multiplied by 100[20].

Treatment

The treatment was described previously[18, 21]. All patients were treated with intensity-modulated radiotherapy. The total dose applied to the pelvis was 45 Gy, administered in 25 fractions over a 5-week period. Following pelvic irradiation, the bilateral parametrium was boosted from 50.4 to 54 Gy.

After adequate tumor regression, high-dose-rate intracavitary brachytherapy was performed once or twice a week using an Ir-192 remote afterloading technique concurrently with pelvic irradiation or parametrial boosting. Before January 2013, the standard prescribed dose for each session of brachytherapy was 6.0 Gy to Point A, with 5 sessions. After January 2013, patients were treated with three-dimensional image-based brachytherapy according to the recommendations of the Groupe Européen de Curiethérapie and the guidelines specified by the European Society for Radiotherapy and Oncology[22]. The details of the cumulative dose are summarized in Table 1.

Chemotherapy consisted of weekly 40 mg/m^2-doses of cisplatin, administered intravenously and accumulating to a total dose of 60 mg.

Follow-up

After completion of radiotherapy, patients were regularly followed up every 2 months for the first year, and every 3 to 4 months thereafter. Besides a routine pelvic examination, the serum levels of tumor markers, namely carcinoembryonic antigens, were examined during each follow-up. A radiographic examination was performed every 6 months. Patients exhibiting symptoms of central-pelvic recurrence underwent a salvage hysterectomy or pelvic exenteration, if feasible. Patients with distant metastasis were treated with systemic chemotherapy.

Statistical analysis

The quantitative differences in the calculated H-scores of the biomarkers between SCC and AC were examined using the Mann–Whitney U test. To examine correlations between the IHC biomarkers and lymph node status or treatment outcome, receiver operating characteristic (ROC) curves were constructed to evaluate the optimal predictive performance among the various IHC and clinical parameters, such as maximum tumor dimension and pretreatment hemoglobulin[23]. In addition, binary logistic regression analysis was performed to determine the independent factors among all IHC biomarkers for predicting the lymph node status. The outcome endpoints were disease-free survival (DFS), distant metastasis-free survival (DMFS), and pelvic relapse-free survival (PRFS), all of which were calculated using the Kaplan–Meier method. The log-rank test and Cox regression analysis were performed to examine the effects of explanatory variables on these endpoints. The stage, age, histology, lymph node status, maximum tumor dimension, baseline hemoglobulin, and predictable IHC markers were included for analysis. Two-tailed tests were used, and $P < .05$ was considered statistically significant. All calculations were performed using SPSS, Version 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Quantitative differences in immunohistochemical biomarkers between the two histological types

All aforementioned IHC biomarkers were retrieved. The quantitative differences in the IHC biomarkers are illustrated in Table 2 and Fig. 1. When using the H-scores of the SCC IHC markers as a reference, increased expression of E-cadherin, calretinin, CAIX, and c-Myc and decreased levels of HIF-1α, VEGF, TNF-α, galectin-9, CCL5, Bax, EGFR, and IGF-1R were found in AC tumors. The mean PD-L1 CPS score was higher in AC than SCC, but no statistical significance existed.

Table 2
Quantitative differences in the expression levels of the immunohistochemical markers between cervical squamous cell carcinoma and adenocarcinoma

Immunohistochemical markers	squamous cell carcinoma (n = 76)	adenocarcinoma (n = 42)	*P value*
HIF-1 α H-score	377.86 ± 127.41	27.07 ± 34.92	< 0.001
CAIX H-score	35.53 ± 39.74	70.56 ± 64.05	0.011
Glut1 H-score	125.13 ± 55.67	130.12 ± 54.84	0.58
VEGF H-score	76.20 ± 58.88	1.51 ± 6.60	< 0.001
EGFR H-score	37.16 ± 45.88	13.95 ± 22.64	0.001
c-Myc H-score	5.78 ± 10.09	28.22 ± 24.12	< 0.001
IGF-1R H-score	40.00 ± 32.33	13.22 ± 21.17	< 0.001
E-cadherin H-score	162.50 ± 54.78	231.90 ± 54.11	< 0.001
Vimentin H-score	12.01 ± 21.86	18.59 ± 40.35	0.09
Calretinin H-score	3.62 ± 9.61	229.88 ± 39.94	< 0.001
Bax H-score	47.13 ± 58.61	2.9 0 ± 7.20	< 0.001
Mcl-1 H-score	123.75 ± 49.24	135.88 ± 41.57	0.57
Bcl-2 H-score	15.91 ± 24.20	39.15 ± 59.31	0.43
TNF-α H-score	57.24 ± 38.10	32.44 ± 38.81	< 0.001
galectin-9 H-score	25.54 ± 24.61	13.71 ± 16.81	0.003
CCL5 H-score	48.08 ± 31.58	3.83 ± 16.30	< 0.001
PD-L1 combined positive score	3.27 ± 4.22	12.58 ± 16.07	0.13
Note: The quantitative differences between H-scores of the biomarkers were examined using the Mann–Whitney U test.			

In summary, cervical SCC exhibited strong expression of the inflammatory, hypoxic, ans angiogenesis markers, whereas the levels of cell to cell adhesion markers were higher in cervical AC. In addition, the Bax levels, an apoptotic activator, were higher in the SCC than in the AC specimens.

Predictive ability s for lymph nodes metastases

Based on the baseline PET/CT, 60 and 15 patients were identified as having pelvic and paraaortic lymph node metastases, respectively. As shown in Supplemental Table 1, ROC curve analysis showed that the H-scores of CCL5 [the area under the ROC curve (AUC): 0.63, P = 0.013], Bcl-2 (AUC: 0.38, P = 0.025), Mcl-1 (AUC: 0.63, P = 0.022) were associated with pelvic lymph node metastasis, whereas the H-scores of Mcl-1 (AUC: 0.67, P = 0.033), TNF-α (AUC: 0.68, P = 0.023), and Glut1 (AUC: 0.71, P = 0.008) were associated with paraaortic lymph node metastasis.

Logistic regression analysis found that a higher CCL5 H-score was a predictor for

pelvic lymph node metastasis [P = 0.004, odds ratio (OR) = 1.018, 95% confidence interval (CI) = 1.006–1.030]. The mean CCL5 H-scores of tumors with and without pelvic lymph node metastasis were 41.90 ± 37.56 and 22.98 ± 28.27, respectively. The analysis also indicated that a higher Glut1 H-score predicted paraaortic lymph node metastasis (P = 0.01, OR = 1.018, 95% CI = 1.004–1.031). The mean Glut1 H-scores of tumors with and without paraaortic lymph node metastasis were 163.00 ± 55.19 and 121.57 ± 55.62, respectively.

Predictive ability for treatment outcomes

In total, 76 patients were alive and 42 patients had died of cancer progression after a median follow-up of 50 months (range, 7–122). Seventy-two patients had no evidence of cancer progression. Thirteen of the 46 patients with tumor progression had pelvic recurrence, 19 had distant metastasis, and 14 had both. None of the 27 patients with pelvic recurrence experienced sole relapse in the lymph nodes. In

summary, 27 patients had local residual or recurrent tumors at primary sites, whereas 33 patients experienced distant metastasis. The 4-year DFS, PRFS, and DMFS for SCC and AC patients were 60% and 57% (P = 0.97), 80% and 67% (P = 0.06), 67% and 70% (P = 0.92), respectively.

Table 3 lists the biomarkers and the AUC if the predictive value was greater than 0.6 or less than 0.4 for any endpoint. The H-scores of 4 IHC markers were associated with the presence of local residual or recurrent tumors, including c-Myc (AUC: 0.65, P = 0.018), Bax (AUC: 0.30, P = 0.002), E-cadherin (AUC: 0.64, P = 0.027), and calreticulin (AUC: 0.63, P = 0.047).

Table 3
Predictive immunohistochemical and clinical parameters and AUC (reported immunohistochemical markers have AUC values ≧ 0.6 or ≦ 0.4)

Variables	cancer progression	local failure	distant metastasis
	AUC / *P* value	AUC / *P* value	AUC / *P* value
c-Myc H-score	0.59 ± 0.05/0.095	0.65 ± 0.06/0.018*	0.51 ± 0.06/0.85
Bax H-score	0.43 ± 0.06/0.19	0.30 ± 0.05/0.002*	0.52 ± 0.06/0.80
TNF-α H-score	0.42 ± 0.05/0.15	0.39 ± 0.06/0.09	0.51 ± 0.06/0.94
Calretinin H-score	0.58 ± 0.06/0.16	0.63 ± 0.06/0.047*	0.51 ± 0.06/0.86
E-cadherin H-score	0.60 ± 0.05/0.078	0.64 ± 0.07/0.027*	0.55 ± 0.06/0.45
galectin-9 H-score	0.43 ± 0.06/0.17	0.39 ± 0.07/0.075	0.41 ± 0.06/0.13
PD-L1 combined positive score	0.54 ± 0.06/0.47	0.60 ± 0.06/0.10	0.39 ± 0.06/0.076
Maximum tumor dimension	0.63 ± 0.05/0.018*	0.64 ± 0.06/0.036*	0.61 ± 0.06/0.07
Pretrement hemoglobulin	0.44 ± 0.06/0.29	0.47 ± 0.07/0.59	0.51 ± 0.06/0.90
SUVmax of primary tumor	0.49 ± 0.05/0.80	0.42 ± 0.06/0.23	0.60 ± 0.06/0.10
Abbreviation: AUC = area under the receiver operating characteristic curve; *SUVmax = maximum standardized uptake value.			

ROC analysis disclosed that none of the other IHC biomarkers, including the hypoxia, cell adhesion, or immunogenicity biomarkers, appeared to be prognostic for distant metastasis or cancer progression. Logistic regression analysis revealed that the existence of pelvic lymph node disease was the sole factor that predicted distant metastasis (P = 0.016, OR = 2.91, 95% CI = 1.22–6.93), and the maximum tumor diameter was the only paramater that predicted cancer progression (P = 0.037, OR = 1.49, 95% CI = 1.03–2.17).

Prognostic factors for DFS, PRFS, and DMFS

To test the prognostic values of the IHC markers, tumors were dichotomized using the median cut-offs of the 4 predictable IHC markers mentioned above. By combining with the clinical parameters, Cox regression analysis was performed. As summarized in Table 4, the results indicated that an E-cadherin H- score > 50% percentile [P = 0.006, hazard ratio (HR) = 2.35, 95% CI = 1.27–4.35] and stage III disease (P = 0.019, HR = 2.15, CI = 1.13–4.07) were two prognostic factors for an inferior DFS. The 4-year DFS of patients with low and high E-cadherin H- scores was 67% and 50% (P = 0.029).

Table 4
Multivariate analysis with Cox regression model for disease-free survival, pelvic relapse-free survival, and distant metastasis-free survival

	Disease-free survival				Pelvic relapse-free survival				Distant metastasis-free survival			
	Univariate model	Multivariate analysis			Univariate model	Multivariate analysis			Univariate model	Multivariate analysis		
Variables	*P*	*P*	HR	95% CI	*P*	*P*	HR	95% CI	*P*	*P*	HR	95% CI
Clinical variables												
AC vs. SCC	0.91				0.06	0.76			0.92			
FIGO stage III vs. IB3-IIB	0.07	0.019*	2.15	1.13–4.07	0.94				0.012	0.50		
Pelvic lymph node					0.89							
positive vs.negative	0.15								0.007	0.01*	2.66	1.26–5.59
Age (continuous)	0.86				0.08	0.10			0.80			
Maximum tumor dimension (continuous)	0.63				0.14				0.33			
Pretreatment hemoglobulin (continuous)	0.12				0.16				0.38			
IHCl biomarkers												
E-cadherin H-score (> 50% percentile vs ≦ 50% percentile)	0.029	0.006*	2.35	1.27–4.35	0.007	0.002*	2.72	1.17–6.35	0.20			
c-Myc H-score (> 50% percentile vs ≦ 50% percentile)	0.95				0.032	0.07	2.50	0.93–6.77	0.61			
Bax H-score (> 50% percentile vs ≦ 50% percentile)	0.50				0.003	0.001*	0.31	0.12–0.76	0.85			
calretinin H-score (> 50% percentile vs ≦ 50% percentile)	0.09	0.10			0.37				0.41			

Abbreviations: HR = hazard ratio; CI- confidence interval; FIGO = International Federation of Gynecology and Obstetrics; AC = adenocarcinoma; ACC = squamous cell carcinoma.

Cox regression analysis disclosed that high E-cadherin and low Bax H-scores were the two predictors of poor PRFS ($P = 0.002$, HR = 2.72, CI = 1.17–6.35 and $P = 0.001$, HR = 0.31, CI = 0.12–0.76, respectively). As depicted in Fig. 2, the 4-year PRFS of patients with tumors with high and low E-cadherin values was 53% and 84% ($P = 0.007$), and the 4-year PRFS of patients with high and low expression of Bax was 88% and 63% ($P = 0.003$), respectively. In SCC patients, the impact of E-cadherin and Bax remained statistically significant (Supplemental Fig. 1).

None of IHC biomarkers were prognostic for DMFS. The major determinant for a low DMFS was pelvic lymph node disease (P = 0.01, HR = 2.66, CI = 1.26–5.59). In multivariate analysis, histology, age, maximum tumor size, and pretreatment hemoglobulin were not identified as independent prognostic factors for the aforementioned endpoints.

Discussion

In cervical cancer, several biomarkers for radiotherapy-based treatment have been validated by patient survival and recurrence data[15, 24]. In view of radioresistance, these biomarkers fall into categories according to biological function including hypoxia, cell proliferation, cell adhesion, immunogenicity, and evasion of apoptosis[15]. There are few IHC comparison studies available for clinical practice which distinguish the differences in CRT-based biomarkers between SCC and AC. This study explored the quantitative differences in a wide range of IHC biomarkers between the two histological types, as well as their roles in determining CRT-based outcomes in patients with locally advanced disease. Herein, we disclosed that cervical SCC exhibited prominent expression of the inflammatory, hypoxic, and angiogenesis markers. In contrast, expression of cell to cell adhesion biomarkers were distinct in cervical AC. Additionally, our results first found that high E-cadherin and low Bax H-scores in tumors were the two determinants for inferior local control. Because expression of high E-cadherin *and* low Bax H-scores were more common in AC than in SCC, it would be interesting to clarify if the profile might contribute to the inferior radiosensitivity reported in cervical AC. Before initiating a novel predictive model for cervical cancer, however, validation studies are required to confirm the findings.

E-cadherin is critical to the maintenance of the epithelial phenotype and provides a structural link between adjacent cellular cytoskeletons, which is important for tissue architecture. Loss of E-cadherin is regarded as a common feature of epithelial–mesenchymal transition (EMT) and is associated with a majority of epithelial cancers[25]. However, many invasive carcinomas infiltrate surrounding tissues as multicellular clusters in which tumor cells remain connected to neighboring tumor cells, which is known as collective invasion[26]. Additionally, recent data demonstrated that a partial EMT resulting in a hybrid epithelial/mesenchymal phenotype with retention of E-cadherin is essential for cancer cell dissemination, and E-cadherin or E-cadherin-based adherens junctions are required for collective invasion and tumor migration[27]. Furthermore, a molecular study disclosed that pretreatment with anti-E-cadherin antibody significantly decreased stromal cell-induced radiation resistance in human prostate cancer[28]. The investigators suggested that cell adhesion molecules such as E-cadherin in cancer cells induce cell survival signals and mediate resistance to cancer treatments such as radiation. The expression of E-cadherin is regulated by genetic and epigenetic mechanisms related to cancer, and its function is modulated by mechanical forces at the junctions, and by multiple signaling pathways[29]. Therefore, integrated molecular studies are required to clarify the biological mechanism by which higher E-cadherin expression in tumors is related to poor radiosensitivity.

Resistance to apoptosis plays an important role in tumors that are refractory to ionizing radiation. Apoptosis regulator Bax, also known as Bcl-2-like protein 4, forms a heterodimer with Bcl-2, and functions as an apoptotic activator. This protein is reported to interact with, and increase the opening of, the mitochondrial voltage-dependent anion channel, which leads to the loss of membrane potential and the release of cytochrome c. In a study investigating a human breast cancer cell line[30], the degree of enhancement of radiosensitivity was dependent on the expression level of Bax. In addition, a superior radiotherapy-based response has been reported in patients with higher Bax expression cervical cancers[31, 32]. On the other hand, Bax is a *p53* primary-response gene, presumably involved in a *p53*-regulated pathway for induction of apoptosis[33]. In an IHC study of cervical carcinomas[34], a total of 66% of the tumors expressed the mutated *p53* protein. The overall survival was better for patients expressing the mutated *p53* protein in the nucleus. Hence, we have been working to clarify the relation between *p53* gene and Bax expression levels in our patients, and their impact on final outcomes. It will be interesting to investigate whether radiation combined with drugs that activate Bax can have a synergistic effect in anticancer treatments by inducing apoptosis in lower Bax expression tumors.

Our study has several limitations. First, this was a retrospective study in a single institution. External validation studies using an independent data set are necessary to confirm these findings. Particularly, future studies should enroll patients prospectively and employ a standardized IHC protocol. Second, the precise molecular pathway that E-cadherin and Bax confers to poor CRT-based local control could not be clarified through association molecular studies, animal experiments, or clinical trials. Finally, the association between DNA sequencing and the protein product should be investigated to understand the comprehensive molecular mechanism of radioresistance and distant metastasis in these patients. Nevertheless, the strengths of this study include the uniform treatment strategies, and wide-ranging analyses of IHC biomarkers. Our findings provide a hint that future studies can clarify the mechanisms related to failure of CRT. In addition, this study initiated a pilot step to enable the tailoring of CRT to the specific biological characteristics of patients with cervical cancers instead of histological types. Our findings disclosed that certain IHC information from cervical tumors might supplement well-known clinical prognostic factors in predicting CRT-based treatment outcomes. Oncologists could then assess the feasibility of personalized therapy for high-risk patients, such as salvage surgery, dose escalation schemes, and a novel combination therapy.

Conclusion

Based on the baseline quantitative analysis of IHC biomarkers in cervical cancers, SCC exhibited strong expression of inflammatory, hypoxic, and angiogenesis biomarkers, whereas the levels of cell adhesion markers were higher in AC. In addition, the Bax intensity in SCC was significantly higher than that in AC. High E-cadherin and low Bax H-scores were two predictable biomarkers associated with high local relapse after definitive CRT. External validation studies are required to verify our findings.

Abbreviations

SCC
squamous cell carcinoma
AC
adenocarcinoma
CRT
chemoradiotherapy
IHC
immunohistochemistry
FDG-PET/CT
fluorodeoxyglucose positron emission tomography/computed tomography
ROC
receiver operating characteristic
AUC
area under the ROC curve
HR
hazard ratio
OR
Odds ratio
DFS
disease-free survival
DMFS
distant metastasis-free survival
PRFS
pelvic relapse-free survival
Glut1
glucose transporter 1 (GLUT1), carbonic anhydrase IX
CAIX
carbonic anhydrase IX
HIF1-α
hypoxia-inducible factor 1-alpha
VEGF
vascular endothelial growth factor,
IGF-R
insulin-like growth factor 1 receptor
Bcl-2
B-cell lymphoma 2
Mcl-1
myeloid cell leukemia 1
TNF-α
tumor necrosis factor-α
CCL5
chemokine ligand 5
PD-L1
programmed cell death protein ligand 1

CPS
combined positive score
EMT
epithelial–mesenchymal transition

Declarations

Ethical approval and Consent to participate: This study was approved by the local institutional review board [CMUH-107-REC3-163].

Consent for publication: All authors agree to publication.

Availability of supporting data: Immunohistochemical data in this study will be available after the consent of all authors.

Competing interest: The authors declare no potential conflicts of interest.

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Figures

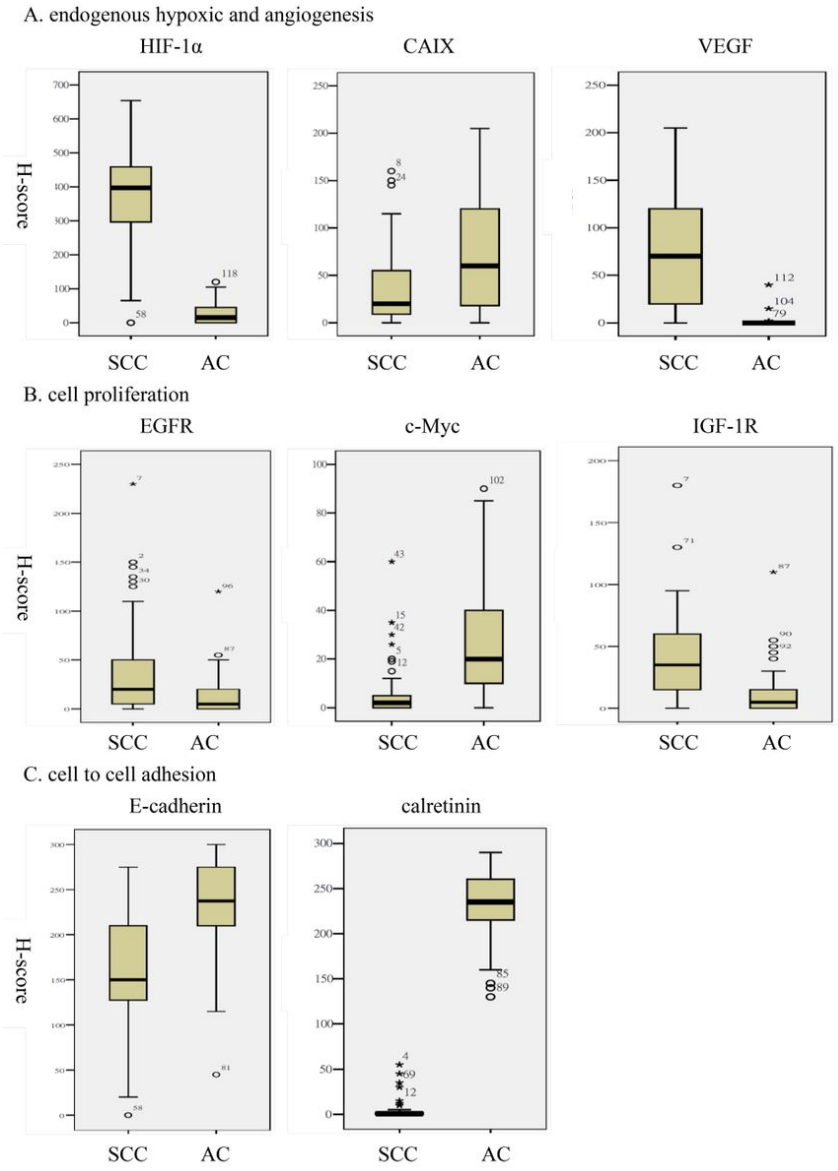

Figure 1

Quantitative differences in the H-scores of the IHC biomarkers between squamous cell carcinoma and adenocarcinoma. (A) endogenous hypoxic and angiogenesis, (B) cell proliferation, (C) cell to cell adhesion

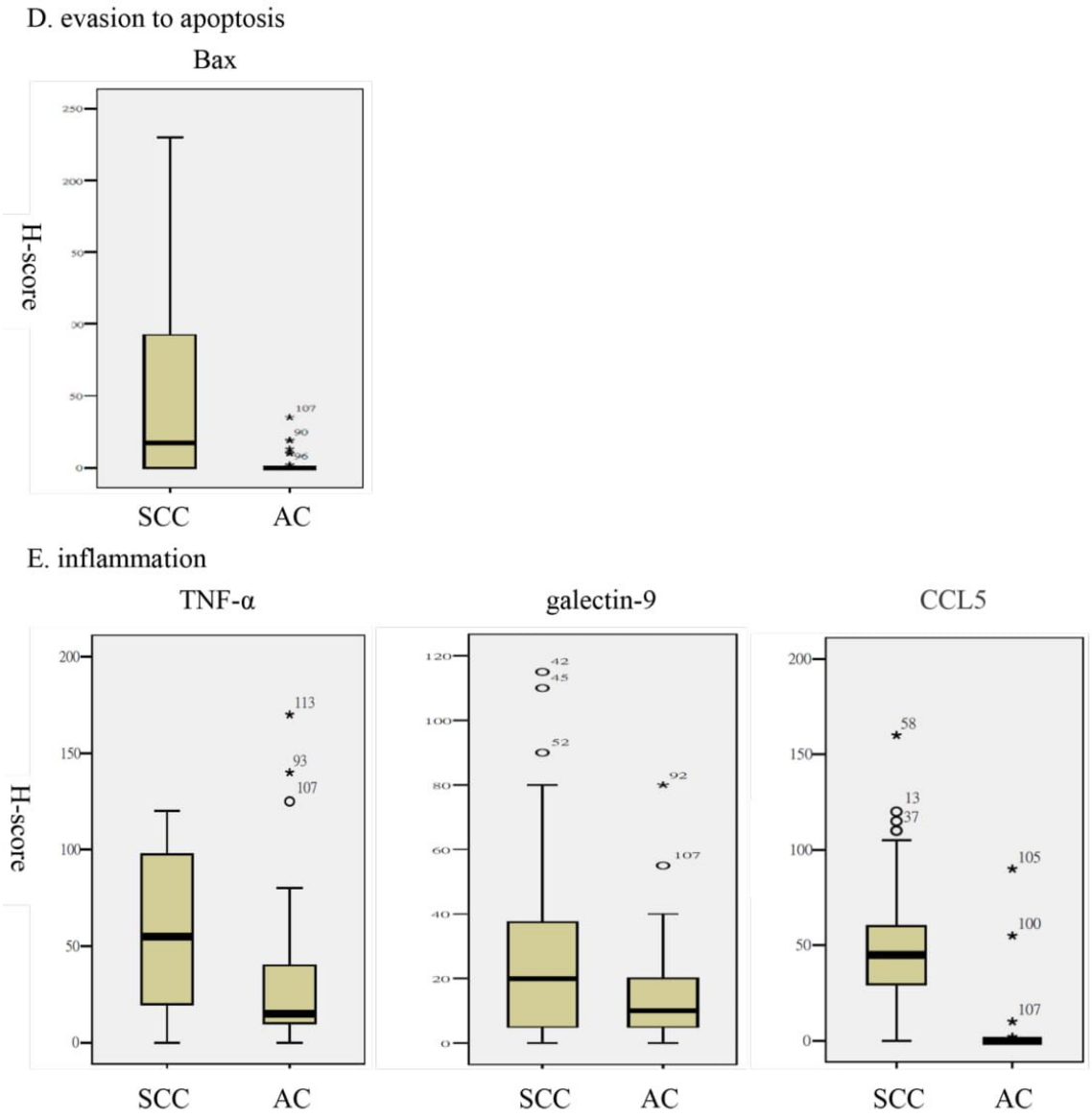

Figure 2

Quantitative differences in the H-scores of the IHC biomarkers between squamous cell carcinoma and adenocarcinoma. (D) evasion to apoptosis, and (E) inflammation biomarkers.

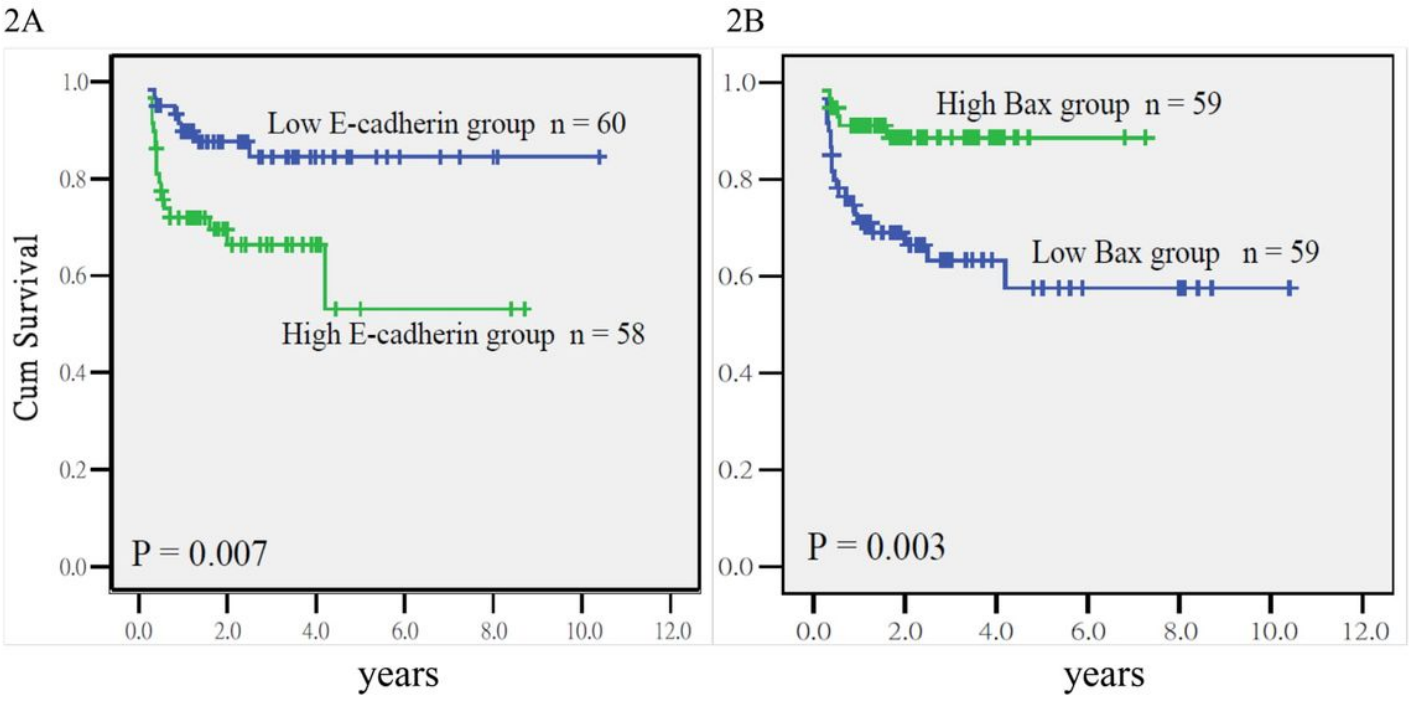

Figure 3

Pelvic relapse-free survival in whole population with tumors in the high E-cadherin group (>50% percentile) and low E-cadherin group (≤50% percentile) (A), and with tumors in the high Bax group (>50% percentile) and low Bax group (≤50% percentile)(B).

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