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Clinical significance of OCT4 and SOX2 protein expression in cervical cancer

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Abstracts

Background: Cancer stem cell markers have become a major research focus because of their relationship with radiation or chemotherapy resistance in cancer therapy. Cancer stem cell markers including OCT4 and SOX2 have been found in various solid tumors. Here, we investigate the expression and clinical significance of OCT4 and SOX2 in cervical cancer.

Methods: To define the clinical significance of OCT4 and SOX2 expression, we performed immunohistochemistry for OCT4 and SOX2 on 305 normal cervical epithelium samples, 289 cervical intraepithelial neoplasia samples, and 161 cervical cancer cases and compared the data with clinicopathologic factors, including survival rates of patients with cervical cancer.

Results: OCT4 and SOX2 expression was higher in cervical cancer than normal cervix (both $p < 0.001$). OCT4 overexpression was associated with lymphovascular space invasion ($p = 0.045$), whereas loss of SOX2 expression was correlated with large tumor size ($p = 0.015$). Notably, OCT4 and SOX2 were significantly co-expressed in premalignant cervical lesions, but not in malignant cervical tumor. OCT4 overexpression showed worse 5-year disease-free and overall survival rates ($p = 0.012$ and $p = 0.021$, respectively) when compared to the low-expression group, while SOX2 expression showed favorable overall survival ($p = 0.025$). Cox regression analysis showed that OCT4 was an independent risk factor (hazard ratio = 11.23, 95 % CI, 1.31 - 95.6; $p = 0.027$) for overall survival while SOX2 overexpression showed low hazard ratio for death (hazard ratio = 0.220, 95 % CI, 0.06–0.72; $p = 0.013$).

Conclusions: These results suggest that OCT4 overexpression and loss of SOX2 expression are strongly associated with poor prognosis in patients with cervical cancer.

Keywords: Neoplastic stem cells, OCT4, SOX2, Prognosis, Survival, Uterine cervical neoplasms

Background

Cervical cancer is one of the most common gynecologic malignancies worldwide and remains a leading cause of cancer-related death for women in developing countries [1]. Such high mortality rates are ascribed to disease recurrence despite cervical resection as well as ineffective treatment options for advanced disease. Radiation therapy is widely employed for advanced cervical cancer, but

radiation resistance is an obstacle for cancer eradication. Radiation resistance of cancer cells is acquired by intrinsic and extrinsic factors including tumor hypoxia, cell cycle and DNA repair and radiation resistance in cancer stem cells (CSCs) [2, 3].

CSCs are a small subpopulation of cancer cells that have stem cell features such as self-renewal and the ability to differentiate into multiple cell types. Radiation therapy or chemotherapy largely eliminates cancer cells, including cervical cancer, but some tumor cells survive and acquire radiation or chemotherapy resistance [4, 5]. These resistant cancer cells are difficult to eradicate, and they show properties of CSCs.

Among CSC markers, octamer-binding transcription factor 4 (OCT4) and sex determining region Y-box 2

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(SOX2) are transcriptional factors involved in the regulation of several target genes including *NANOG*, *Fgf4* and *Utf1*, as well as *OCT4* and *SOX2* [6–10]. *OCT4* belongs to the POU (Pit-Oct-Unc) transcriptional factor family and plays a key role in stem cell pluripotency and differentiation by determining the fate of embryonic stem cells [11]. *OCT4* expression in cancer stem-like cells is associated with self-renewal and tumorigenesis via regulation of its target genes [12]. *OCT4* expression has been shown to be correlated with poor tumor differentiation and metastasis, as well as poor prognosis in colon, pancreas and lung cancer [13–15]. *SOX2*, a member of the SRY-related HMG-box (SOX) family of transcription factors, stimulates the reprogramming of adult cells into induced pluripotent stem cells and maintains stem cell-like properties in cancer by complexing with other stem cell markers such as *NANOG* and *OCT4* [9]. *SOX2* expression was reported to be correlated with tumorigenesis, chemoresistance and maintenance of stem cell-like phenotype in cancer cells [16, 17]. In addition, *SOX2* has been shown to be highly expressed in premalignant lesions such as squamous dysplasia and carcinoma in situ in lung [18]. Prior studies suggest *OCT4* and *SOX2* have a key role of tumorigenesis and prognosis of cancer. However, the prognostic significance of *OCT4* and *SOX2* is not clearly defined in cervical premalignant and malignant lesion. In this study, we investigated the clinical significance of *OCT4* and *SOX2* expression in cervical neoplasia.

Methods

Patient selection

A total of 450 patients with cervical cancer and cervical intraepithelial neoplasia (CIN) were collected from patients who enrolled at Gangnam Severance Hospital, Yonsei University College of Medicine in Seoul, Korea and the Korea Gynecologic Cancer Bank through Bio & Medical Technology Development Program of the Ministry of Education, Science and Technology, Korea between 1996 and 2010. One hundred sixty-one paraffin-embedded specimens of cervical cancer, 289 CIN and 305 matched normal tissues were included in the study. Medical records were obtained to review patient data including age, cancer stage, tumor differentiation, cell type, tumor size, lymphovascular space invasion (LVSI) and lymph node (LN) metastasis. Cervical cancer was staged according to the International Federation of Gynecology and Obstetrics (FIGO) stage and histologically classified and graded according to World Health Organization (WHO) grade. Patients with surgical indications underwent radical hysterectomy with pelvic and aortic lymph node dissection. Concurrent chemoradiation therapy was added in cases with risk factors such as LN metastasis, parametrial invasion and positive resection margin. Inoperable patients underwent

radiation or chemoradiation therapy. Tissue samples and medical records were obtained with informed consent of all patients and approval of the local research ethics committee (approval no. 3-2010-0030; Seoul, South Korea). This study was additionally approved by the Office of Human Subjects Research at the National Institute of Health.

Tissue microarray construction and immunohistochemistry

Tissue microarrays (TMAs) were constructed from 450 patients with primary invasive cervical cancer or CIN, as well as 305 matched non-adjacent normal epithelial tissues. After hematoxylin and eosin slides were reviewed by a pathologist, areas containing each category were indicated by marking them. Four 1-mm punches were then taken from the corresponding regions of the paraffin blocks and transplanted into a recipient paraffin block using a tissue arrayer (Pathology Devices, Westminster, MD).

For immunohistochemical staining, all paraffin-embedded sections were cut at 5- μ m thickness followed by deparaffinization through xylene and dehydration with graded ethanols. Antigen recovery was performed in heat-activated antigen retrieval pH 6 (Dako, Carpinteria, CA) for *OCT4* and *SOX2*, and then specimens were incubated with 3 % H_2O_2 for 10 min. Non-specific binding was blocked with protein block (Dako) for 20 min at room temperature. The sections were incubated with rabbit polyclonal anti-*OCT4* antibodies (Abcam, Cambridge, MA; Cat. #ab19857) at 1:250 for 30 min or with rabbit monoclonal anti-*SOX2* antibodies (Cell Signaling, Danvers, MA; Cat. #3579) at 1:500 for 2 h, respectively. Subsequently, antigen-antibody reaction was detected with EnVision + Dual Link System-HRP (Dako) and visualized with DAB+ (3, 3'-Diaminobenzidine; Dako). Tissue sections were lightly counterstained with hematoxylin and then examined by light microscopy. Negative controls (substitution of primary antibody with TBS) were run simultaneously. Positive controls included testicular seminoma and lung squamous cell carcinoma for *OCT4* and *SOX2* [19] antibodies, respectively.

Quantitative evaluation of immunostaining

Immunohistochemically stained slides were digitized at $\times 20$ magnification utilizing an Aperio Scanscope CS (Aperio, Vista, CA, USA). Images were reviewed using an online software application, Digital Image Hub (SlidePath, Dublin, Ireland). Once the areas were annotated, they were sent for automated image analysis utilizing TissueIA (SlidePath's Tissue IA system, version 3.0, Dublin, Ireland). Within Tissue IA, an algorithm was developed to quantify *OCT4* and *SOX2* expression levels. The staining intensity of *OCT4* and *SOX2* was categorized as 0 (no staining), 1+ (weak), 2+ (moderate) and 3+ (strong). The overall immunohistochemical score

(histoscore) was expressed as the percentage of positive cells multiplied by their staining intensity (possible range, 0–300) [20].

Statistical analysis

Histoscores were compared using one-way ANOVA test and independent *t*-test. The immunohistochemical cut-off for high expression of tumor markers was determined through receiver operating characteristic (ROC) curve analysis. The sensitivity and (1 - specificity) for discrimination of dead and alive was determined for each immunohistochemistry (IHC) score and plotted, thus generating a ROC curve. The cut-off value was established to be the point on the ROC curve where sum of sensitivity and specificity was maximized. Kaplan-Meier survival analysis was performed to determine the association of OCT4 and SOX2 expression with survival, and the survival curves were compared between groups using log-rank tests. Multivariate analyses of hazard ratio for death were performed using Cox proportional hazards regression. Chi-square test was used to evaluate the association between OCT4 and SOX2. Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL). A value of $p < 0.05$ was considered statistically significant.

Results

Clinicopathologic characteristics of cases

Table 1 presents the patients' clinicopathologic characteristics. Of 161 patients with cervical cancer, 118 patients were stage IIA or less and 43 patients were stage IIB or higher. The mean age was 43.3 years (range, 19–83 years). The tumor sizes ranged from 0.2 to 12.0 cm (mean, 2.8 cm). The histopathology included 131 squamous cell carcinoma, 16 adenocarcinoma, 7 adenosquamous and 7 other types (3 small cell carcinomas, 2 neuroendocrine and 2 mixed cell types). Patients with cervical cancer were evaluated for survival analysis and the mean follow-up time of surviving patients was 54.3 months (range, 1–179). Fifteen patients (9.3 %) died during the follow-up period.

OCT4 and SOX2 protein expression

Expression of OCT4 and SOX2 was evaluated by IHC in cervical neoplasia and cancer specimens. Subsequently, we performed analysis of both markers using quantitative image analysis software. Representative IHC images of OCT4 and SOX2 are presented in Fig. 1. OCT4 expression was observed primarily in the nucleus with limited cytoplasm expression, while SOX2 was restricted to the nucleus (Fig. 1). Only nuclear staining was considered OCT4- and SOX2-positive.

Table 1 Patient clinicopathologic characteristics

	Frequency	%
Age	43.3 ^a	
Diagnostic category		
Normal	305	40.4
Low grade CIN	59	7.8
High grade CIN	230	30.5
Cancer	161	21.3
FIGO stage		
< IIA	118	73.3
> IIB	43	26.7
Tumor differentiation ^b		
Well	2	1.3
Moderate	112	71.8
Poor	42	26.9
Cell type		
SCC	131	81.4
AD	16	9.9
Other	14	8.7
Tumor size		
≤ 4 cm	112	69.6
> 4 cm	49	30.4
LVSIF ^c		
No	86	56.2
Yes	67	43.8
LN metastasis ^d		
No	115	74.2
Yes	40	25.8
HPV test in CIN ^e		
Negative	21	14.2
Positive	127	85.8

CIN cervical intraepithelial neoplasia, FIGO International Federation of Gynecology and Obstetrics, SCC squamous cell carcinoma, AD adenocarcinoma, LVSIF lymphovascular space invasion, LN lymph node, HPV human papillomavirus

^amean value

^bcalculated based on 156 cases with available tumor differentiation information

^ccalculated based on 153 cases with available LV invasion information

^dcalculated based on 155 cases with available LN metastasis information

^ecalculated based on only 148 cases of CIN with available HPV test data

Of the cancer specimens, 92 of 161 cancers (57.1 %) had high expression of OCT4 (histoscore > 200) and 125 of 161 cancers (77.6 %) had high expression of SOX2 (histoscore > 30). Association of OCT4 and SOX2 expression with clinicopathologic characteristics in cervical cancer is summarized in Table 2. OCT4 and SOX2 expression was significantly different depending on diagnostic category ($p < 0.001$). OCT4 overexpression was associated with lymphovascular space invasion ($p = 0.045$), whereas loss of SOX2

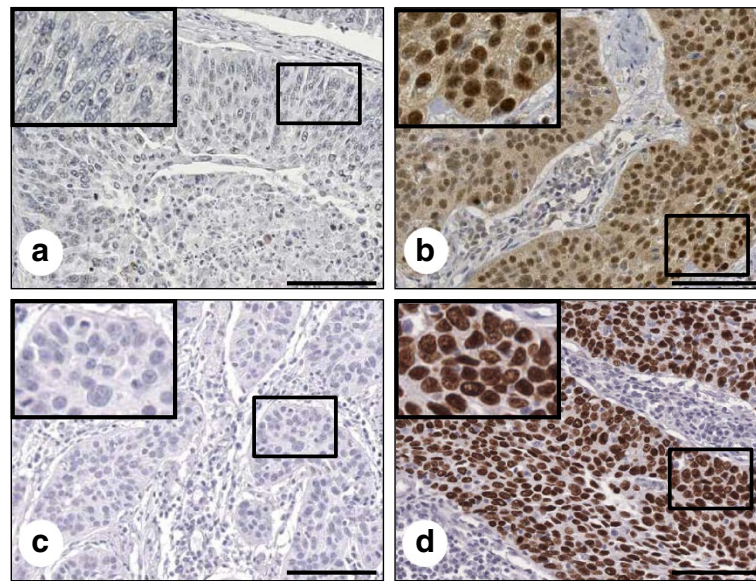


Fig. 1 OCT4 and SOX2 expression in formalin-fixed, paraffin-embedded cervical cancer tissues. Representative immunohistochemical image of OCT4 negative (a) and positive (b), SOX2 negative (c) and positive (d). Insets show high magnification of areas indicated with boxes. Scale bar: 100 μ m

expression was correlated with large tumor size ($p = 0.015$). There were no other correlations between OCT and SOX2 expression and clinicopathologic characteristics.

We next examined the association between OCT4 and SOX2 expression, Chi-squared distribution was used in malignant and premalignant lesions. In premalignant cervical lesions, SOX2 expression presented a significant correlation with OCT4 ($p = 0.004$), while there was no association between OCT4 and SOX2 in malignant tumors ($p = 0.543$; Table 3).

Prognostic significance of OCT4 and SOX2 expression

Five-year disease-free and overall survival rates were analyzed through the Kaplan-Meier plots as shown in Fig. 2. In survival analysis with OCT4 expression, 22 recurrences and 13 deaths occurred in 83 cases of OCT4 high expression, while 7 recurrences and 3 deaths were observed in 65 cases of low expression. The 5-year disease-free and overall survival rates were 89.2 and 95.4 % in low OCT4 expression and 73.5 and 84.3 % in high OCT4 expression. OCT4 overexpression was associated with shorter disease-free and overall survival than the low expression group ($p = 0.012$ and $p = 0.021$, respectively) (Fig. 2a and d). In survival analysis of SOX2, there were 20 recurrences and 8 deaths in 125 high-expression patients, while 8 recurrences and 7 deaths occurred in 36 low-expression patients during the 5-year follow-up period. The 5-year disease-free and overall survival rates were 77.8 and 80.6 % in cases with low SOX2 expression and 84.0 and 93.6 % in case with high SOX2 expression cases. High expression of SOX2 was associated with better overall survival than low expression

($p = 0.025$) (Fig. 2e). When survival of patients with expression of high OCT4/low SOX2 was compared with survival of patients with low OCT4/high SOX2, Kaplan-Meier analysis revealed a significant difference in disease-free and overall survival ($p = 0.016$ and $p < 0.001$, respectively; Fig. 2c and f).

Cox proportional multivariate analysis of relationships between prognostic variables and survival are shown in Table 4. FIGO stage was an independent survival factor for both disease-free and overall survival analysis ($p < 0.001$ and $p = 0.041$, respectively). OCT4 overexpression showed independent poor overall survival with a hazard ratio of 11.23 ($p = 0.027$), while high expression of SOX2 presented better disease-free and overall survival compared to low expression, as shown in Table 4 ($p = 0.019$ and $p = 0.013$, respectively).

Discussion

OCT4 and SOX2 are important transcriptional factors involved in maintenance of pluripotency and self-renewal in cancer stem cells, aberrant expression of OCT4 and SOX2 might contribute to carcinogenesis in various cancers [15, 21, 22]. Radioresistance is important in the treatment and prognosis of cervical cancer and it is known to be associated with cancer stem cells [3]. This study examined the clinical correlation and prognostic significance of stemness-related OCT4 and SOX2 protein expression assessed by IHC in premalignant and malignant cervical tumors. The results demonstrate that OCT4 and SOX2 protein expression is elevated in premalignant and malignant cervical tumors compared to normal cervix and this finding is consistent with a

Table 2 Association between clinicopathologic characteristics and OCT4 or SOX2 expression

	OCT4		SOX2	
	Mean HistoScore (95 % CI)	<i>p</i> value	Mean HistoScore (95 % CI)	<i>p</i> value
Diagnostic category		<0.001		<0.001
Normal	113.3 (105.1–121.4)		36.5 (32.3–40.8)	
Low-grade CIN	197.6 (177.4–217.7)		40.0 (28.8–51.2)	
High-grade CIN	219.0 (211.3–226.8)		91.7 (79.9–103.5)	
Cancer	208.5 (196.7–220.3)		105.4 (91.8–119.1)	
FIGO stage		0.498		0.529
< IIA	205.7 (191.6–219.7)		108.1 (92.1–124.2)	
> IIB	215.1 (192.6–237.6)		98.3 (71.5–125.1)	
Tumor differentiation		0.438		0.112
Well + moderate	203.8 (187.7–219.8)		112.8 (94.4–131.1)	
Poor	213.4 (195.3–231.4)		90.1 (69.0–111.3)	
Cell type		0.450		0.060
SCC	205.8 (192.4–219.2)		111.7 (96.4–127.0)	
Other	217.2 (190.8–243.6)		78.3 (69.0–111.3)	
Tumor size		0.868		0.015
≤ 4 cm	208.7 (194.2–223.2)		116.5 (100.1–132.8)	
> 4 cm	206.5 (185.5–227.6)		80.3 (56.3–104.2)	
LVSI		0.045		0.106
No	195.3 (175.8–214.8)		115.5 (95.4–135.6)	
Yes	220.1 (205.3–234.9)		91.8 (71.2–112.5)	
LN metastasis		0.206		0.879
No	202.6 (187.1–218.0)		105.6 (88.6–122.7)	
Yes	221.0 (199.7–242.3)		103.1 (75.6–130.6)	
HPV test in CIN		0.292		0.907
Negative	246.8 (223.7–215.7)		124.9 (81.5–168.7)	
Positive	227.9 (215.7–240.1)		127.6 (110.5–144.6)	

SCC squamous cell carcinoma, AD adenocarcinoma, LVSI lymphovascular space invasion, LN lymph node, HPV human papillomavirus

previous study [23]. OCT4 was an independent poor survival factor but SOX2 showed as a favorable prognostic factor.

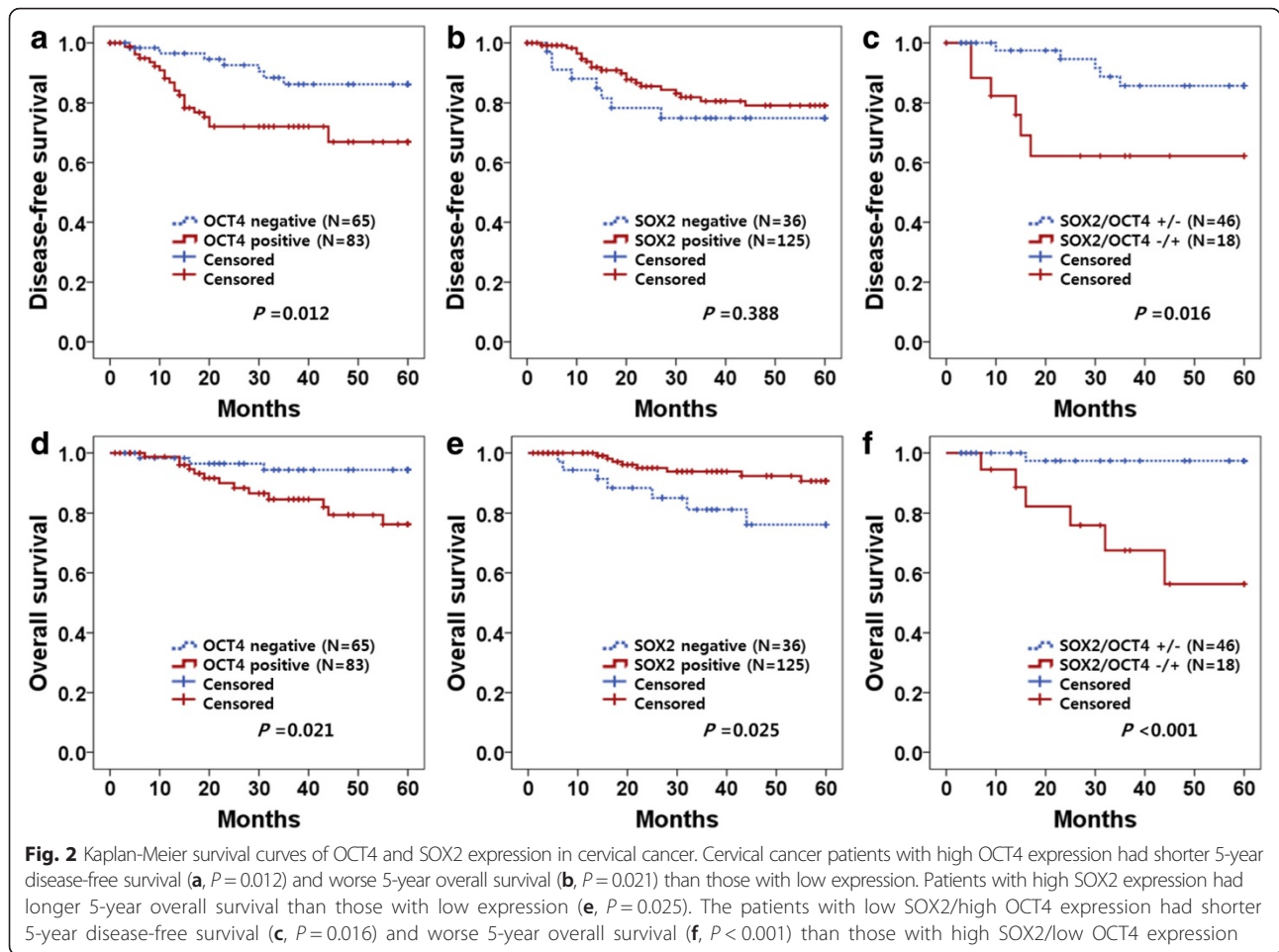
In this study, OCT4 protein was observed clearly in the nucleus and partially in the cytoplasm. Similar to

Table 3 Association of OCT4 and SOX2 expression in CIN and cervical cancer patients

	OCT4 expression			<i>p</i> value
	No.	Low (%)	High (%)	
CIN				0.004
SOX2 Low (-)	55	29 (53.1)	26 (46.9)	
SOX2 High (+)	234	62 (26.3)	173 (73.1)	
Cancer				0.543
SOX2 Low (-)	36	15 (41.7)	21 (58.3)	
SOX2 High (+)	125	54 (43.1)	71 (56.9)	

CIN cervical intraepithelial neoplasia

our findings, OCT4 has been reported in the cytoplasm as well as in the nucleus in previous studies [24, 25]. This staining pattern may arise from the presence of an OCT4 isoform. OCT4 is known to have two isoforms, OCTA and OCTB. OCT4A is observed in the nucleus and OCT4B is observed in the cytoplasm in prostate and cervical cancer [24, 26]. Because OCT4 is a transcriptional regulator, the active form of OCT4 is always located in the nucleus. For this reason, we focused our automated digital image analysis on OCT4 protein expression in the nucleus only. Notably, OCT4 expression increased during cancer progression but within cancers, it was not correlated with known prognostic factors, such as stage, LN metastasis or tumor size. Nonetheless, it showed high hazard ratio of death in multivariate analysis. In previous published results, OCT4 expression was associated with unfavorable prognosis



showing poor tumor differentiation, tumor invasion and metastasis in the lungs, stomach, esophagus and oral cavity [13, 25, 27, 28]. Although there have been limited reports on the association between OCT4 and prognosis in cervical cancer, Shen et al. reported that OCT4 expression was associated with radiation-resistance and unfavorable survival in locally advanced squamous cell carcinoma [29]. That study further showed OCT4 overexpression in the radiation resistance group, but it was not associated with high risk prognostic factors including FIGO stage and tumor size, which is similar to our results. It is interesting

that OCT4 is associated with poor survival without correlation to known prognostic factors and even disease-free survival. As a stem cell related protein, OCT4 expression can be related more with overall survival than with disease-free survival which is possibly more related with residual tumor after resection. Further research is required to clarify the association between OCT4 and high-risk prognostic factors.

SOX2 is known to play an important role in regulating the cell cycle, DNA repair and self-renewal in stem cells [30]. It is associated with tumorigenesis, chemoresistance and maintenance of stem cell-like property in cancer cells,

Table 4 Multivariate survival analysis of the association between prognostic variables and survival in cervical cancer patients

Variables	Disease-free survival		Overall survival	
	HR [95 % CI]	<i>P</i> value	HR [95 % CI]	<i>P</i> value
FIGO stage (\geq IIB)	8.67 [2.77–27.11]	<0.001	4.33 [1.06–17.72]	0.041
Tumor size (>4 cm)	1.16 [0.47–2.84]	0.739	1.79 [0.54–5.92]	0.340
LN metastasis	1.72 [0.57–5.23]	0.334	1.60 [0.40–6.41]	0.500
OCT4+	3.75 [1.24–11.55]	0.117	11.23 [1.31–95.64]	0.027
SOX2 +	0.47 [0.18–1.20]	0.019	0.22 [0.06–0.72]	0.013

FIGO International Federation of Gynecology and Obstetrics, HR hazard ratio, LN lymph node, CI confidence interval

which suggests poor overall survival [16, 31, 32]. High expression of SOX2 was reported to be associated with a lack of cell differentiation and to contribute cell migration and invasion in cervical cancer cell line [33]. In addition, Shen et al. showed that SOX2 is highly expressed in patients with radiation resistance and predicts poor survival [29]. In contrast, SOX2 expression was associated with prolonged survival in the current study. These discrepancies might be explained by the lack of standardized methodology, different standards of interpretation or differences in studies' patient populations. Similar to our study, Wilbertz et al. reported that SOX2 gene amplification and protein expression are associated with favorable survival outcomes in squamous cell lung cancer [34]. In addition, a recent meta-analysis reported that SOX2 expression presents a positive prognosis in non-small cell lung cancer [35]. Previously, SOX2 overexpression was reported to be associated with favorable prognosis in squamous cell lung carcinoma, but was correlated with poor survival in adenocarcinoma [34–36]. Notably, the poor survival associated with SOX2 expression that was reported in GI tract cancer mostly pertained to adenocarcinoma [37–39]. Cervical cancer consists of squamous cell carcinoma followed by adenocarcinoma and our data comprised 80.7 % squamous cell carcinoma and 14.8 % adenocarcinoma. Further research is required to clarify the prognostic significance of SOX2 in cervical cancer and variation in prognosis according to cell type.

Premalignant cervical lesion demonstrated significant correlation between OCT4 and SOX2, while malignant lesion did not present an association between OCT4 and SOX2. The lack of a correlation between OCT4 and SOX2 in malignant lesions has not been explained clearly because OCT4 and SOX2 are known to work cooperatively and self-regulate themselves via the OCT4/SOX2 complex in embryonic stem cells [6, 8]. However, in the current cancer tissue samples, OCT4 and SOX2 were associated with opposite effects on survival and lose their association in cervical cancer, as well. Similar to our results, no correlation between OCT4 and SOX2 was reported in cervical cancer [23]. In addition, Li et al. also reported that OCT4 and SOX2 were not co-expressed and also showed different survival outcomes in lung cancer tissue samples [40]. Furthermore, overexpression of SOX2 inhibited the activity of OCT4 promoter in embryonal carcinoma cells [41]. OCT4 and SOX2 are known to function cooperatively through the OCT4/SOX2 complex, but OCT4, SOX2 and Nanog have been reported to form individual complexes with nucleophosmin to control stem cell fate determination [42]. In previous study, we also observed a similar phenomenon that Nanog expression in precancerous cervical tissue was correlated with Tc1a and pAkt but this relationship lost in cancerous tissue [43]. Considering previous

results and our contradictory survival data, OCT4 and SOX2 might function independently or inhibit activity during tumor progression, and eventually lose their connection in cervical cancer.

Conclusions

In conclusion, this study investigated the immunohistochemical expression of OCT4 and SOX2 in large number of cervical cancer patients by means of image analysis for IHC scoring. OCT4 and SOX2 showed high expression in premalignant and malignant cervical tumors. Co-expression of OCT4 and SOX2 was observed in premalignant tumors, but no association was observed in malignant cervical tumors. OCT4 high expression showed poor disease-free survival and overall survival while SOX2 high expression showed favorable overall survival in patients with cervical cancer. Cox regression analysis confirmed that OCT4, and SOX2 expression was an important prognostic indicator in cervical cancer. OCT4 was associated with poor prognosis, while SOX2 showed favorable prognosis. Our findings suggest further investigation into OCT4 and SOX2 as biomarkers in cervical cancer.

Abbreviations

AD: adenocarcinoma; CI: confidence interval; CIN: cervical intraepithelial neoplasia; CSC: cancer stem cell; FIGO: International Federation of Gynecology and Obstetrics; H&E: hematoxylin and eosin; HPV: human papillomavirus; HR: hazard ratio; IHC: immunohistochemistry; LN: lymph node; LVSI: lymphovascular space invasion; OCT-4: octamer-binding transcription factor 4; SOX2, sex determining region Y-box 2; ROC: receiver operating characteristic; SCC: squamous cell carcinoma; TMA: tissue microarray; WHO: World Health Organization.

Competing interests

The authors declare that there is no conflict of interest.

Authors' contributions

BWK, J-YC, J-HK and SMH conceived of the study and devised the experimental design. SMH designed and build the tissue microarrays. BWK, HC, CHC and KY performed experiments. BWK, HC, CHC, J-YC, J-HK and SMH performed data analysis for experiments or clinical records. BWK, HC and J-YC drafted the final version of the manuscript and figure legends. J-HK and SMH revised the figures, added critical content to the discussion and were responsible in revising all portions of the submitted portion of the manuscript. All authors read and approved the final manuscript.

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