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Sex-determining region Y-box 2 (SOX2) expression predicts poor prognosis in human ovarian carcinoma

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Summary

Sex-determining region Y-box 2 (SOX2) is proposed to be a key transcription factor in embryonic stem cells. The known roles of SOX2 in development and cell differentiation suggest that it is relevant to the aberrant growth of tumor cells. Thus, SOX2 may play an important role in tumor progression. However, its clinical significance in human ovarian carcinoma has been uncertain until recently. The aim of the present study was to clarify the clinical role of SOX2 expression in ovarian carcinoma. Immunohistochemical staining of 540 human ovarian carcinoma samples for SOX2 was performed using tissue microarray. The associations among SOX2 expression and clinical factors (diagnosis, tumor grade, International Federation of Gynecology and Obstetrics stage, and response to chemotherapy), overall survival, and disease-free survival were analyzed. We observed SOX2 expression in 15% of the ovarian carcinoma samples. Use of the Fisher exact test suggested that SOX2 expression was associated with high-grade carcinoma ($P = .009$), especially high-grade serous carcinoma ($P = .048$); International Federation of Gynecology and Obstetrics stage (II-IV, $P = .005$); and malignant mixed müllerian tumors ($P = .048$). SOX2 expression was also associated with decreased disease-free survival durations ($P = .035$; log-rank test). Our results showed that SOX2 expression may be a potential marker of related to tumor recurrence, as implicated to its role in cancer stem cells.

Keywords

Sex-determining region Y-box 2 (SOX2); Immunohistochemistry; Ovarian carcinoma; Prognosis

1. Introduction

Ovarian cancer is the most lethal gynecologic malignancy, as it was responsible for about 14,000 deaths in 2010 according to the American Cancer Society [1]. Despite continued development of surgical techniques and meticulously designed chemotherapy regimens, the overall 5-year survival rate in patients with ovarian cancer only increased from 37% to 46% over 30 years [1]. Usually, currently used surgery techniques and/or chemotherapy regimens

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can mostly or partly alleviate the tumor burden, but residual therapy-resistant cancer cells may cause a relapse at the primary site and/or metastasize to secondary sites to initiate new tumors.

Over the past decade, the hypothesis that cancer arises from a small subset of cancer cells often referred to cancer stem cells (CSCs) or tumor initiating cells (TICs) that are capable of generating entire tumor has become a great interest in the cancer research community. The presence of CSCs in tumors may herald virtually unlimited proliferation, self-renewal, and high resistance to chemotherapy and/or radiotherapy [2]. Therefore, these cells are proposed to be a subpopulation of tumor cells critical to tumor development, progression, metastasis, and recurrence [3].

The transcription factor sex-determining region Y-box 2 (SOX2), which belongs to group B of the SOX family, is encoded by a single-exon gene on chromosome 3q26.3-q27 [4]. SOX2 is highly expressed in embryonic stem cells and considered a key transcription factor [5]. SOX2 can aid the reprogramming of adult cells into induced pluripotent stem cells, indicating it has critical roles in cellular fate determination, differentiation, and proliferation [6,7]. The known roles of SOX2 in development and cell differentiation suggest that it is relevant to the aberrant growth of tumor cells and may be related to CSCs. Researchers have found SOX2 expression in several solid tumors, including melanoma [8]; brain tumor [9], digestive tract [10-11], breast [12], liver [13], pancreatic [14], and prostate [15], and lung carcinoma [16]. SOX2 is thought to maintain cellular proliferation potential via the Notch signaling pathway [17] and modulation of cyclin D1 expression [12].

In human primary ovarian cancer, the clinical role of SOX2 expression has remained unknown until recently. Because SOX2 may play an important role in tumor progression, we evaluated its expression via immunohistochemical staining of human ovarian carcinoma samples in comparison with normal fallopian tube and ovarian tissue samples using tissue microarray and analyzed the associations among SOX2 expression and clinical factors (diagnosis, tumor grade, International Federation of Gynecology and Obstetrics [FIGO] stage, and chemotherapy response), overall survival (OS), and disease-free survival (DFS).

2. Materials and methods

2.1. Patients and clinicopathologic data

Human ovarian carcinoma samples obtained from 540 patients who underwent initial surgery at The University of Texas MD Anderson Cancer Center from January 1st, 1990, to February 22nd, 2007, were analyzed. Relevant clinical data were obtained via retrospective review of the patients' medical files. These data included demographic information, diagnosis, tumor grade, disease stage, ascites and serum cancer antigen (CA) 125 level, chemotherapy regimen, and response to clinical treatment or chemotherapy. Their follow-up information was updated through April 30th, 2011, by reviewing their medical records and the U.S. Social Security Death Index. Also, 20 normal fallopian tube and 9 normal ovarian tissue samples obtained from patients with uterine tumors were analyzed at same time. Use of tissue blocks and chart reviews was approved by the MD Anderson Institutional Review Board.

The patients' histopathologic diagnoses were based on World Health Organization criteria [18]. Nonserous carcinomas were graded according to the Gynecologic Oncology Group criteria [19-21], and serous carcinomas were graded using a two-tier system (low and high grade) [22]. Disease was staged using the FIGO system [23].

To analyze responses to chemotherapy, the patients were categorized as responders or nonresponders. The responders were those who experienced complete clinical remission with a normal serum CA 125 level (normal range, 0-35 U/ml) after chemotherapy for histologically or cytologically diagnosed carcinoma and who had a treatment-free interval of at least 6 months [24]. Nonresponders were patients with progressive or recurrent disease. Progressive disease was defined as carcinoma that progressed without any observed remission after the initiation of treatment, whereas recurrent disease was defined as carcinoma detected after a period of clinically documented remission shorter than 6 months [25].

The OS duration was defined as the time from the date of first biopsy to the date of death caused by ovarian carcinoma or the last follow-up date. The DFS duration was defined as the time from the date of first biopsy to the date of recurrence. Recurrence was indicated clinically by the appearance of new lesions or an increase in the serum CA 125 level to more than twice the upper limit of normal range [26].

2.2. Immunohistochemical analysis

Tissue microarray blocks of ovarian carcinoma were made as described previously [27]. Tissue microarray slides were stained immunohistochemically in accordance with the Biocare Medical manufacturer's protocol (Biocare Medical, Concord, CA). After tumor sections were deparaffinized and rehydrated, antigen retrieval in them was performed using a universal decloaker (UD1000M; Biocare Medical) in an autoclave at 121°C for 5 minutes and blocked using Peroxidized 1 endogenous peroxidase blocker (PX968; Biocare Medical) at room temperature for 10 minutes. Nonspecific binding was blocked with Background Sniper (BS966M; Biocare Medical) for 15 minutes at room temperature. The slides were incubated with a primary mouse monoclonal antibody against SOX2 (MAB2018, 1:1000; R&D Systems, Minneapolis, MN) overnight at 4°C, with a biotin-labeled secondary antibody for 20 minutes (Universal Goat Link, GU600H; Biocare Medical), and finally with 4plus HRP 1000 Universal for 20 minutes (HP604; Biocare Medical). After being stained with 3,3'-diaminobenzidine chromogen (DB801L; Biocare Medical) for 5 minutes, the sections were counterstained with hematoxylin, dehydrated, and mounted. Negative controls were constructed by replacing the primary antibody with phosphate-buffered saline. A seminoma sample was used as a positive control for SOX2. All of the controls yielded satisfactory results.

The immunohistochemically stained slides were independently analyzed for SOX2 expression by two gynecologic pathologists (J.L. and J.Z.). The degree of staining was half-quantified using a five-score grading system of 0 to 4: 0, samples without SOX2-positive cells; 1, samples with less than 5% SOX2-positive cells; 2, samples with 5-25% SOX2-positive cells; 3, samples with 26-50% SOX2-positive cells; 4, samples with >50% SOX2-positive cells. In tumor cells, only nuclear staining was classified as SOX2-positive expression. For statistical analysis, the cases were classified as negative (0% SOX2-positive cells) or positive (>0% SOX2-positive cells) for SOX2 expression.

2.3. Statistical analysis

The Fisher exact test and logistic regression analysis were used to evaluate the association of SOX2 expression with clinical factors. One-way analysis of variance for multiple comparisons was performed to evaluate the association of SOX2 expression with response to chemotherapy and the grade of serous/endometrioid carcinoma. The Kaplan-Meier method and log-rank test were used to estimate OS and DFS rates. To adjust for the confounding effects of age at diagnosis, tumor grade, response to clinical treatment, diagnosis, and FIGO stage, unconditional logistic regression analysis was performed using

multiple covariates to evaluate the association of SOX2 expression with OS and DFS. *P* levels less than .05 were considered statistically significant. The SPSS (version 17.0; SPSS, Chicago, IL) and Stata (version 8.0; StataCorp, College Station, TX) software programs were used for statistical analyses.

3. Results

3.1. Patient characteristics

The mean age of the patients was 59 years (range, 20-92 years). At the initiation of our study, 81 patients were alive without ovarian carcinoma, 43 were alive with ovarian carcinoma, 296 had died of ovarian carcinoma, and 107 had died of an unrelated or unknown ovarian carcinoma; 13 lost follow-up and were excluded from the OS analysis. The median OS duration was 4.4 years (95% confidence interval [CI], 3.7-5.2 years), and the OS rate was 62% (95% CI, 60-64%) at 3 years, 46% (95% CI, 44-48%) at 5 years, and 34% (95% CI, 32-36%) at 10 years.

Among 540 patients, 67 did not have relapses, 221 did have relapses, and 206 had disease progression; 46 patients lost follow-up or had unknown serum CA 125 levels. We included only patients with and without disease relapse in the DFS analysis. The median DFS duration was 1.4 years (95% CI, 1.1-1.7 years), and the DFS rate was 35% (95% CI, 32-38%) at 3 years, 28% (95% CI, 25-31%) at 5 years, and 23% (95% CI, 20-26%) at 10 years.

3.2. SOX2 expression and localization

SOX2 immunoreactivity was located in the nuclei of normal epithelial cells. Thirteen (65%) of the normal fallopian tube samples were positive for SOX2 expression (Fig. 1A). In these 13 samples, SOX2-positive epithelial cells were sparse and scattered in 9 samples (69%) and focal in 4 samples (31%). None of the normal ovarian tissue samples were positive for SOX2 expression (Fig. 1B).

In tumor cells, SOX2 expression was mainly located in the nuclei. Seventy-nine (15%) of the 540 ovarian carcinoma samples were positive for expression of SOX2. Among these 79 cases, the immunohistochemical score was 1 in 47 patients (59%), 2 in 11 patients (14%), 3 in 8 patients (10%), and 4 in 13 patients (16%). A majority of samples with staining pattern in score 1 showed scattered positive cells. Thirty-six cases of high-grade serous carcinoma were positive with score 1. Representative examples of SOX2 staining in ovarian carcinoma samples were shown in Figs. 1C-1H. The SOX2 expression rate in the ovarian carcinoma samples was much lower than that in the normal fallopian tube samples ($P < .001$).

3.3. Association of SOX expression with clinicopathologic variables

We summarized the results of immunohistochemical staining of the ovarian carcinoma samples for SOX2 based on the patients' clinicopathologic characteristics (Table 1). SOX2-positive expression was associated with malignant mixed müllerian tumors ($P = .048$), high-grade carcinoma ($P = .009$), and FIGO stage II-IV tumors ($P = .005$). Because of different grading systems, we analyzed the association of SOX2 expression with ovarian serous and endometrioid carcinoma grades separately. There was no SOX2-positive expression in low-grade serous and endometrioid carcinoma. SOX2-positive expression was found in 14% (58/410) of high-grade serous carcinoma. There was significant association between SOX2-positive expression and high-grade serous carcinoma ($P = .048$). Whereas, we observed no significant associations between SOX2 expression status and different endometrioid carcinoma grades ($P = .271$) (data not shown).

The association of SOX2 expression with response to chemotherapy was shown in Table 2. Of the 540 patients, 518 (96%) received chemotherapy. Among these patients, 386 (75%) received postsurgical cisplatin-based treatment either alone or with other drugs, 76 patients (15%) received presurgical cisplatin-based treatment, and 7 patients (1%) received other forms of chemotherapy (melphalan, GOG-182, paclitaxel [Taxol], 5-fluorouracil plus folinic acid, and Taxol plus cyclophosphamide [Cytosan]). We observed no significant associations between chemotherapy response and SOX2 expression.

3.4. Association of SOX2 expression with OS and DFS

The association of SOX2 expression with OS and DFS durations and rates at 3, 5, and 10 years was shown in Table 3. Patients with SOX2 expression had shorter median DFS durations than did patients without SOX2 expression ($P = .035$) (Fig. 2). We found no association between SOX2 expression and OS duration ($P = .640$). Furthermore, we observed no significant associations among the different immunohistochemical scores for SOX2 expression (0, 1, 2, 3, or 4) and OS ($P = .810$) or DFS ($P = .088$) duration.

Multivariate Cox proportional hazards regression analysis indicated that the SOX2 expression status was not an independent predictor of OS or DFS duration in patients with ovarian carcinoma when challenged by the covariates disease stage, response to clinical treatment, age at diagnosis, and tumor grade (Table 4). Overall, FIGO stage was significantly associated with OS and DFS. Patients with FIGO stage II-IV tumors had a higher risk of death (odds ratio [OR], 3.87; 95% CI, 1.79-8.39; $P = .001$) and relapse (OR, 3.18; 95% CI, 1.59-6.35; $P = .001$) than the patients with FIGO stage I tumors. Also, patients whose age at diagnosis was at least 60 years had a higher risk of death (OR, 1.44; 95% CI, 1.13-1.84; $P = .003$) than did patients younger than 60 years of age.

4. Discussion

In the present study, we found that SOX2 expression in ovarian carcinoma tissues was associated with high-grade carcinoma (especially high-grade serous carcinoma) and FIGO stage II-IV disease. Moreover, patients with ovarian carcinoma having SOX2 expression had shorter DFS durations than did patients without this expression. These results indicated that SOX2 may be a potential marker of ovarian CSCs related to tumor recurrence, which is similar to its role in regarding embryonic stem cells.

Although SOX2 is expressed in a variety of solid tumors, the roles of SOX2 expression in clinical value have been controversial. Our results were in accordance with those of the majority of studies that SOX2 expression in cancer tissue was significantly associated with poor prognosis and aggressive tumor characteristics, such as a high histologic grade, poor clinical survival duration, high TNM stage [13,28]. Some researchers have even proposed that SOX2 is an oncogene [29] that can promote proliferation and tumorigenesis of human lung squamous cell carcinoma [16] and breast cancer [12]. However, in gastric cancers, SOX2 expression is frequently downregulated and can inhibit cell growth via cell-cycle arrest and apoptosis. Loss of SOX2 expression in gastric cancer cells may be related to gastric carcinogenesis and poor prognosis [10]. Patients having gastric cancer with high SOX2 expression levels have exhibited lower incidences of lymph node metastasis, deeper invasion, later clinical stages, and better prognoses than those with low SOX2 expression levels [30]. Furthermore, in squamous cell lung cancer cases, SOX2 gene amplification and protein overexpression have been associated with improved outcome [31]. Therefore, SOX2 may behave differently in patients with different cancers.

Regarding localization of SOX2, researchers have demonstrated that both the nuclear and cytoplasmic localization of SOX2 may depend on the antibody used in

immunohistochemical or immunofluorescence staining [9,32]. Consistent with most reports of SOX2 expression in the literature, our data demonstrated SOX2 expression was located mainly in the nuclei of ovarian carcinoma cells and nonspecific cytoplasmic localization of SOX2 was observed when the dilution of the antibody (MAB2018; R&D Systems, Minneapolis, MN) was 1:1000. Because it is a transcription factor, SOX2 activation is defined by stronger nuclear staining and detection in a higher proportion of cells in nuclei than in cytoplasm [29]. Sequestering SOX2 in the cytoplasm is a potent mechanism of negatively affecting its activity. The SOX2 protein expression level must be maintained in the nucleus to sustain its pluripotency [33]. Therefore, in our study, we considered only nuclear staining for SOX2 to be an indicator of positivity for SOX2 expression.

Recently, based on clinicopathologic and genetic molecular analyses, researchers have begun regarding epithelial cells in the ovaries and fallopian tubes as originators of ovarian carcinoma [34]. For this reason, we analyzed SOX2 expression in normal ovarian and fallopian tube tissue samples as well as the tumors. To our surprise, 65% of the normal fallopian tube samples had SOX2 expression, a rate much higher than that in the ovarian carcinoma samples. However, we observed no SOX2 expression in normal ovarian tissue samples. These results may indicate that SOX2 has different functions in normal ovarian and fallopian tube tissue during ovarian tumorigenesis; if so, its specific biologic mechanisms require further study.

In conclusion, our study revealed an association between SOX2 expression and advanced ovarian cancer, implying that SOX2 expression is closely related to poor clinical outcome in patients with ovarian cancer and a potential marker of ovarian CSCs related to tumor recurrence, similar to its role in cancer stem cells.

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Fig. 1. Immunohistochemical staining for SOX2 in normal ovarian, normal fallopian tube, and ovarian carcinoma samples. A, Epithelial cells in a normal fallopian tube tissue exhibiting nuclear staining for SOX2. B, A normal ovarian tissue sample negative for SOX2 expression. C, A high-grade serous carcinoma sample negative for SOX2 expression. D, A high-grade serous carcinoma sample with more than 50% of the tumor cells exhibiting expression of SOX2. E, A high-grade endometrioid carcinoma sample positive for SOX2 expression. F, High-grade serous carcinoma cells with high levels of SOX2 expression in the nuclei. G, Malignant mixed müllerian tumor cells exhibiting high levels of nuclear staining for SOX2. H, Clear cell carcinoma sample exhibiting expression of SOX2.

Fig. 2.

Kaplan-Meier survival curves for patients with ovarian carcinoma positive and negative for SOX2 expression. A, OS (n = 527). B, DFS (n = 288).

Table 1
Associations of SOX2 expression with clinicopathologic factors

Characteristic	SOX2 expression			<i>P</i> ^a
	Total no. of patients	Negative (%)	Positive (%)	
Histologic type				.048
High-grade serous carcinoma	410	352 (86)	58 (14)	
Low-grade serous carcinoma	24	24 (100)	0 (0)	
Endometrioid carcinoma	40	34 (85)	6 (15)	
Clear cell carcinoma	18	15 (83)	3 (17)	
Transitional cell carcinoma	12	9 (75)	3 (25)	
Malignant mixed müllerian tumor	15	9 (60)	6 (40)	
Undifferentiated carcinoma	13	11 (85)	2 (15)	
Others ^b	8	7 (88)	1 (13)	
FIGO stage				.005
I	34	34 (100)	0 (0)	
II-IV	506	427 (84)	79 (16)	
Grade				.009
Low	32	32 (100)	0 (0)	
High	508	429 (84)	79 (16)	
Ascites level				.674
No	61	54 (89)	7 (11)	
50 ml	41	37 (90)	4 (10)	
>50 ml	325	277 (85)	48 (15)	
Unknown	113	93 (82)	20 (18)	
Response to clinical treatment				.707
No	66	58 (88)	8 (12)	
Yes	432	368 (85)	64 (15)	
Unknown	42	35 (83)	7 (17)	
Age at diagnosis				.468
<60 years	273	230 (84)	43 (16)	
60 years	267	231 (87)	36 (13)	
Serum CA 125 level				.216
800 U/ml	187	162 (87)	25 (13)	
>800 U/ml	208	170 (82)	38 (18)	
Unknown	145	123 (85)	22 (15)	

^aFisher exact test.

^bMucinous carcinoma (six cases) and mixed-type carcinoma (two cases).

Table 2
Correlation of expression of SOX2 with response to chemotherapy

Response to chemotherapy	SOX2 expression		
	Total no. of patients	Negative (%)	Positive (%)
Responders ^a			
Postsurgery cisplatin-based regimen ^b	205	172 (84)	33 (16)
Presurgery cisplatin-based regimen ^c	21	15 (71)	6 (29)
Other regimen	3	2 (67)	1 (33)
Unknown regimen	2	2 (100)	0 (0)
Nonresponders ^a			
Postsurgery cisplatin-based regimen ^b	181	158 (87.3)	23 (12.7)
Presurgery cisplatin-based regimen ^c	55	49 (89)	6 (11)
Other regimen	4	4 (100)	0 (0)
Unknown regimen	1	1 (100)	0 (0)
No chemotherapy	22	19 (86)	3 (14)
Unknown response	46	39 (85)	7 (15)
Total	540	461 (85)	79 (15)

^aResponders versus nonresponders ($P = .104$).

^bResponders versus nonresponders ($P = .345$).

^cResponders versus nonresponders ($P = .059$).

P values were calculated using one-way analysis of variance.

Table 3
Association of SOX2 expression with OS and DFS

Variable	OS (n = 527)		DFS (n = 288)	
	SOX2-negative	SOX2-positive	SOX2-negative	SOX2-positive
No. of patients	448	79	245	43
Median survival duration, years (95% CI)	4.4 (3.6-5.3)	4.7 (2.5-7.0)	1.5 (1.1-1.8)	1.1 (0.9-1.2)
Survival rate (95% CI)				
3 years	0.62 (0.60-0.65)	0.57 (0.51-0.63)	0.38 (0.34-0.41)	0.19 (0.13-0.25)
5 years	0.46 (0.43-0.48)	0.49 (0.42-0.55)	0.31 (0.28-0.34)	0.06 (0.03-0.08)
10 years	0.35 (0.32-0.37)	(<0.12-<0.28)	0.25 (0.22-0.28)	N/A (<<0.12)
<i>p</i> ^a	.640		.035	

^aLog-rank test.

Table 4
Multivariate analysis of OS and DFS in the study patients

Variable	OS		DFS	
	OR (95% CI)	<i>P</i> ^a	OR (95% CI)	<i>P</i> ^a
SOX2 expression (negative versus positive)	1.03 (0.74-1.45)	.853	0.80 (0.54-1.17)	.240
Response to clinical treatment (no versus yes)	1.05 (0.74-1.51)	.772	0.94 (0.63-1.42)	.783
Histologic type ^b	0.99 (0.89-1.09)	.810	0.91 (0.81-1.03)	.135
Grade (low versus high)	1.14 (0.65-1.99)	.646	1.46 (0.80-2.66)	.224
FIGO stage (I versus II-IV)	3.87 (1.79-8.39)	.001	3.18 (1.59-6.35)	.001
Age at diagnosis (<60 versus ≥ 60 years)	1.44 (1.13-1.84)	.003	1.31 (0.97-1.75)	.074

^aDerived from unconditional logistic regression analysis with multiple covariates.

^bHigh-grade serous carcinoma versus low-grade serous carcinoma versus Endometrioid carcinoma versus clear cell carcinoma versus transitional cell carcinoma versus malignant mixed müllerian tumor versus undifferentiated carcinoma versus others (mucinous carcinoma and mixed-type carcinoma).