Sex-determining region Y box-containing genes: regulators and biomarkers in gynecological cancers

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ABSTRACT
Sex-determining region Y box-containing genes are transcription factors with roles in multiple biological processes, including cell differentiation, proliferation, and apoptosis. Sex-determining region Y box-containing genes have also been shown to act as regulators and biomarkers in the progression of many different cancers, including gynecological cancers such as ovarian, cervical, and endometrial cancer. In this review, we summarize the contrasting regulatory roles of Sex-determining region Y box-containing genes in different gynecological cancers, as promoters with high expression levels or as suppressors with low expression levels. Expression levels of Sex-determining region Y box-containing genes were also identified as biomarkers of clinical features, including International Federation of Gynecology and Obstetrics stage, histopathologic grade together with disease-free survival, and treatment efficacy in patients with gynecological cancers. An understanding of the mechanisms whereby Sex-determining region Y box-containing genes regulate the progression of gynecological cancers will aid in the development of novel diagnostic and therapeutic strategies, while analysis of Sex-determining region Y box-containing expression levels will help to predict the prognosis of patients with gynecological cancers.

KEYWORDS
Sex-determining region Y box-containing gene; gynecological cancer; regulator; biomarker; clinical feature; progression

Introduction
Sex-determining region Y box-containing (SOX) genes encode regulatory transcription factors that can act as tumor suppressors or promoters in carcinogenesis. SOX genes were shown to be involved in the development of various cancers, including breast, lung, hepatocellular, and gastrointestinal cancers. Due to the increased morbidity and mortality of gynecological cancers (GCs) year by year, the roles of SOX genes in GCs, including ovarian (OC), cervical (CC), and endometrial cancer (EC) become a recent focus of researches. Studies investigating the relationship between aberrant expression of SOX genes and the development of GCs found that some SOX genes with high expression level were expected to act as oncogenic regulators which promoting the progression of GCs, while those with low expression level were regarded as suppressors with the opposite effects. Furthermore, regulating expression level of SOX genes in certain cancer cell lines could influence cell proliferation and apoptosis in vitro but without known mechanisms. Additionally, analysis of abnormal SOX genes expression in patient samples contributed to the detection of early GC lesions. Expression level of SOX genes was also considered as biomarkers associated with clinical features in patients with GCs, including International Federation of Gynecology and Obstetrics (FIGO) stage, histopathologic grade, and disease-free survival (DFS), as well as treatment response. Understanding the mechanisms whereby SOX genes regulate the progression of GCs is therefore valuable and may facilitate the development of novel diagnostic, therapeutic, and prognostic strategies which aimed at improving the prognosis of women with GCs. This review provides a systematic summary of the SOX genes’ roles in the progression of GCs, and highlights future directions for research.

Classification and functions of SOX genes

Classification of SOX genes
SOX genes encode a conserved group of regulatory transcription factors comprising about 414-amino-acid
polypeptides with a highly conserved high-mobility group (HMG) box\textsuperscript{20,21}. This box encodes around 79-amino-acid DNA-binding domain, with two L-shaped arms that can bind to ATTTGTT or related DNA sequence motifs in the minor groove by recognizing the sequence 5′-(A/T)(A/T)CAA(A/T)-3′, resulting in widening of the minor groove, unwinding of the DNA helix, and DNA bending\textsuperscript{22}. This domain was first identified in SRY, as a crucial factor involved in determining male sex of mammals. Genes that encode proteins containing an HMG domain with at least 50% amino acid similarity to the SRY HMG domain are considered as SOX genes\textsuperscript{23}. To date, mammalian genomes have been found to include approximately 30 different SOX genes, which can be classified into 10 subgroups (A–J) based on the degree of homology of the amino acid sequence inside the HMG domain, the presence of conserved motifs outside the HMG domain, and their full-length structures (Figure 1)\textsuperscript{24–26}. In this review, we summarize the roles of some GC-related SOX genes, including SOX1, SOX2, SOX3, SOX4, SOX7, SOX8, SOX9, SOX11, SOX14, SOX15, SOX17, and SOX18.

Functions of SOX genes

The activities of the various subgroups of SOX genes are multi-faceted. Fundamentally, SOX genes are involved in sex determination and the development of the testis, prostate, endothelial cells, and the vascular, lymphatic, and nervous systems during vertebrate embryonic development\textsuperscript{24,27}. However, the multiple functions of SOX genes in the development of these various systems alerted researchers to their potential roles in the development of diseases, especially cancers\textsuperscript{12}. Most recent studies of SOX genes focused on their involvement in gastric cancer, lung cancer, hepatocellular carcinoma, and prostate cancer\textsuperscript{2,28,29}. Studies indicated that most SOX genes played their roles in these cancers through the Wnt/β-catenin signaling pathway, as the so-called ‘canonical’ Wnt pathway mediated by β-catenin\textsuperscript{12,13}. Activation of the Wnt/β-catenin signaling pathway decreases phosphorylation of β-catenin in the cytoplasm and increases β-catenin transfer into the nucleus. Consequently, it activates the nuclear complex of β-catenin/T cell factor/lymphoid enhancer factors, and enhances expression level of cell cycle-related molecules such as cyclin-D1 and c-Myc\textsuperscript{12,30}. Thus, a discussion of how SOX genes act in the progression of GCs via different signaling pathways, especially the Wnt/β-catenin signaling pathway is presented below.

**SOX genes in GCs**

Numerous studies currently focus on the roles of SOX genes in GCs. In this review, OC, CC, and EC are selected to be representative GCs due to their high incidence and mortality. In general, SOX genes have been identified as regulators influencing the progression of GCs, as well as biomarkers of clinical features. Clinical, cellular, and animal experiments have shown that some SOX genes act as oncogenes while others act as tumor suppressor genes in these three cancers (Figure 2). General depiction of the expression level of SOX...
genes in GCs and mechanisms of how they perform effectively are shown in Figure 3.

**SOX genes in OC**

OC is one of the three most common cancers in women, with an estimated 22,240 new diagnoses and 14,070 deaths in the United States in 2018. Due to lack of specific symptoms and reliable screening methods, approximately 70% of patients with OC are diagnosed at advanced stage with metastasis beyond the ovary, which contributes to its high mortality. Thus, there is an urgent need to find novel diagnostic biomarkers for detecting OC at a premalignant stage.

SOX genes as clinical biomarkers for OC

Regarding their roles as clinical biomarkers, multiple studies analyzed the relationships between SOX gene expression level in OC samples and clinical features, including FIGO stage, histopathologic grade, and DFS (Table 1). Researchers identified SOX1, SOX7, and SOX11 as tumor suppressor genes, with low expression level in patient samples due to aberrant CpG island hyper-methylation or unclear mechanisms. Low expression level of these genes in cancerous tissues or serum was detected more frequently in patients with more advanced stage, higher grade, more aggressive tumor behavior, and shorter recurrence-free survival (RFS), while higher level was associated with the opposite clinical features. These results suggested that analyzing SOX gene expression might be a good biomarker for predicting the prognosis of patients.

However, in addition to tumor suppressor role of SOX genes, SOX2 was shown to play dual roles in OC, with high expression level identified as a poor prognostic biomarker in some cases, but as a favorable factor in other cases. On one hand, high SOX expression level in fallopian tube epithelium was exploited as a biomarker for OC screening, especially in BRCA1 or BRCA2 mutation carriers or in women with serous OC in high grade. High expression level of SOX2 in patient samples was also shown to be related to high grade, advanced FIGO stage, and decreased DFS. On the other hand, Pham et al. demonstrated that high expression level of SOX2 was a favorable biomarker indicating longer DFS and overall survival (OS) in patients with stage II–IV high-grade...
serous OC among 215 cases of OC. Other researchers also affirmed the good prognostic effects of SOX2 in 570 samples from patients with ovarian serous cystadenocarcinoma. The mechanisms responsible for these different effects of SOX2 may be due to various factors, such as feedback mechanisms of SOX2 expression, interactions between SOX2 in the cytoplasm and nucleus, or differences between patient spectra and measuring methods. These flexible and bidirectional roles of SOX2 suggest that SOX2 could be a ‘double-edged sword’ depending on how scientists choose to utilize it. However, analyzing expression level of SOX gene in tissues is still generally considered to be a valuable approach for predicting clinical features in patients with OC.

SOX genes as regulators in the progression of OC

In addition to their role as clinical biomarkers, accumulating evidence from in vitro studies indicated that SOX genes acted as vital regulators in the progression of OC, including in cell proliferation, apoptosis, and metastasis (Figure 4). SOX2 was considered as an oncogene at the cellular level, and up-regulation of SOX2 in OC cell lines promoted cell proliferation and tumor sphere formation via hypoxic treatment and overexpression of the intracellular domain of Notch. Moreover, transduction of SOX2 into OC cell lines also enhanced resistance to cell apoptosis through overexpression of the anti-apoptotic gene BCL2 and simultaneous down-regulation of the pro-apoptotic genes PUMA/BBC3 and NOXA/PMAIP1. Overexpression of SOX2 also accelerated cell migration by down-regulating E-cadherin and up-regulating vimentin expression. These results at the cellular level were not as the same as the results based on clinical samples, which suggested that in vitro studies could not imitate the environment in the body completely. And more animal studies are needed to clarify the role of SOX2 in the progression of OC. In addition to SOX2, up-regulation of SOX4 in OC cell lines by the long non-coding RNA BRM promoted cell proliferation, migration, and invasion via an unknown mechanism. More researches are therefore also needed to investigate the mechanisms and potential of SOX4 in OC.

SOX7 and SOX11, which are considered as tumor suppressor genes at both the clinical and molecular level,
inhibited the progression of OC. Low level of SOX7 together with increased cyclooxygenase-2 and cyclin-D1 were detected in tissues from patients with epithelial OC, especially in patients with advanced serous cystadenocarcinoma\textsuperscript{10}. This suggested that SOX7 might regulate cell proliferation by influencing the Wnt/\beta-catenin signaling pathway. Transduction of SOX11 into OC cell lines was also reported to inhibit cell proliferation, though the mechanism remained unclear\textsuperscript{18}. These members of the SOX gene family all contribute to the progression of OC, and their various

\begin{table}[h]
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\begin{tabular}{|l|l|l|}
\hline
\textbf{SOX genes} & \textbf{Potential clinical implication in gynecological cancers} & \textbf{References} \\
\hline
\textbf{OC} & Methylation of SOX1 was a clue to detect premalignant OC and predict recurrence as well as worse survival & 17, 35 \\
SOX1 & ① High expression level of SOX1 was exploited as a biomarker for screening OC  
② High expression level of SOX1 implied advanced FIGO stage together with shorter DFS  
③ SOX1 could improve cell resistance to chemo-treatment  
④ High expression level of SOX1 indicated favorable DFS and OS in stage II to IV high-grade serous OC  
SOX7 & High expression level of SOX7 was regarded as a good prognostic marker & 10  
SOX11 & High expression level of SOX11 was associated with improved recurrence-free survival & 18, 40  
\hline
\textbf{CC} & Hyper-methylation of SOX1 was an effective biomarker that can discriminate early lesions of CC from normal tissues & 14, 41-46  
SOX1 & ① High expression level of SOX2 was associated with higher grade along with poorer differentiation  
② Higher expression level of SOX2 indicated bigger tumor size, more metastasis and invasion as well as shorter OS and RFS  
③ High expression level of SOX2 implied radiation resistance and unfavorable prognosis  
④ High expression level of SOX2 indicated favorable DFS together with OS compared with low expression of it  
SOX2 & SOX4 increased cell resistance to cisplatin and contributed to progression of CC & 52  
SOX8 and SOX17 & Hyper-methylation of SOX8 along with SOX17 was detected more frequently in CIN3+ lesions & 53  
SOX9 & ① SOX9 enhanced CC cell resistance to chemo-treatment  
② High methylation level of SOX9 could be used as an early screening marker and indicated poor behavior of CC  
\hline
\textbf{EC} & Analysis of high methylation index of SOX1 in patients' tissues was demonstrated as a potential method for detection of EC hidden in atypical hyperplasia & 56, 57  
SOX1 & ① Low level of SOX2 due to hyper-methylation of CpG island upstream in promoter region indicated type II serous or clear cell adenocarcinoma and short survival  
② High expression level of SOX2 was associated with high grade, tumor metastasis and local recurrence which implied poor outcome  
SOX2 & Low or absent expression level of SOX7 in tissues was associated with high grade EC & 19  
SOX9 & High expression level of SOX9 inhibited cell proliferative but implicated high histologic grade due to a feedback system & 59  
SOX17 & ① Loss of SOX17 indicated more advance stage, higher grade and shorter recurrence free-survival of patients with EC  
② High expression level of SOX17 in tissues indicated that patients might own increased sensitivity and toxicity to cisplatin & 21, 60, 61  
\hline
\end{tabular}
\caption{Abnormal expression of SOX genes and their potential clinical implications in gynecological cancers}
\end{table}
mechanisms warrant more detailed investigation. These results also suggested that controlling the expression of certain SOX genes may be a promising therapeutic target in the near further.

**SOX genes influence treatment efficacy in OC**

In terms of treatment, overexpression of SOX2 was shown to enhance chemoresistance of OC cell lines to cisplatin through activating the expression of the drug efflux transporter genes ABCB1 and ABCG2. Knockdown of SOX2 in cell lines accordingly decreased chemoresistance to cisplatin via down-regulating expression of ABCB1, ABCG2, and ABCC6. These results were accordance with those of researches on the roles of SOX2 based on OC cell lines, but not with researches based on patients’ samples in other cases. Further simultaneous *in vitro* and *in vivo* researches into SOX2 are therefore needed to develop its role as a promising new therapeutic target for patients with OC.

**SOX genes in CC**

CC is a common GCs considered to be an accidental endpoint of persistent infections with certain types of human papillomavirus (HPV), especially HPV16 and HPV18. Some investigators found that SOX2 regulated HPV16 transcription by inhibiting activity of its long control region and finally decreasing expression of the E6 and E7 oncogenes in CC carcinogenesis. Furthermore, mounting evidence
implicated other members of the SOX genes family in the regulation of CC progression (Figure 5) and identified their roles as biomarkers for predicting the prognosis of patients with CC (Table 1).

**SOX genes as screening biomarkers and prognostic factors in CC**

Analysis of the aberrant expression level of SOX1, SOX8, SOX9, SOX14, and SOX17 were identified as a novel early screening method for distinguishing between early CC lesions and normal tissues by methylated-CpG island recovery assay (Table 1). Higher methylation level of these genes was accompanied by more severe cervical squamous cell lesions\(^{14,41-46,55}\). Moreover, the sensitivity and specificity of this early screening method was increased by the combined detection of the methylation level of more than one SOX gene, such as SOX1 and SOX14, or SOX8 and SOX17\(^{53,65}\). Further studies are therefore needed to determine if analyzing the expression level of all SOX genes combined might increase their sensitivity as screening biomarkers for CC.

In terms of prognostic biomarkers, SOX2 was identified as a dual-effect gene, with its expression level having different implications for clinical features in different studies. Four studies detected high level of SOX2 in tissues from CC patients and concluded that high SOX2 expression was associated with higher grade, poorer differentiation, advanced stage, and poorer survival\(^{7,48-50}\) (Table 1). However, the opposite effects were observed in other studies. For example, Kim et al.\(^{47}\) detected SOX2 expression in tissue samples from normal cervical epithelium, cervical intraepithelial neoplasia, and CC by immunohistochemistry, and found that high expression level of SOX2 were correlated

![Figure 5](image-url) Roles of SOX genes (SOX1, SOX2, SOX4, SOX9, SOX14 and SOX18) in the progression of CC. Oncogenes included SOX4, SOX2 and SOX18 in red color. SOX1 was regarded as suppressor gene in blue color. SOX9 and SOX14 played bidirectional roles in purple color. Small green arrows indicate promotion and small red arrows indicate inhibition. There were few studies of SOX4 and SOX18 yet. P21\(^{\text{WAF1/CIP1}}\) is a main kind of cyclin-dependent kinase inhibitor in cell cycle.
with favorable DFS and OS. The unknown mechanisms behind this phenomenon may help to account for the roles of SOX2 in OC at the clinical level. Nevertheless, more researches are needed to elucidate the precise mechanisms responsible for the effects of SOX2.

**SOX genes regulate progression of CC**

Similar to their roles in OC, numerous studies investigated the involvement of SOX genes in regulating the progression of CC (Figure 5). Consistent with an oncogenic role, some studies found that transduction of CC cell lines with SOX2, SOX4, and SOX18 promoted cell proliferation, metastasis, and invasion. Specifically, endogenous overexpression of SOX2 or SOX4 in CC cell lines drove the cell cycle from G0/G1 to S stage by up-regulation of cyclinE2, minichromosome maintenance protein 10 (MCM10), and well protein kinase. Overexpression of SOX2 in CC cell lines also promoted metastasis and invasion by augmenting the expression of epithelial–mesenchymal transition-promoted molecules, such as vimentin, β-catenin, and Snail. However, the action of SOX2 at the cellular level did not necessarily reflect the same behavior in clinical samples because of the absence of the body’s internal environment. SOX18 promoted the development of CC without clear mechanisms, so further studies are needed to clarify the mechanisms responsible for the oncogenic role of SOX18.

In contrast, SOX1 is considered as a tumor suppressor gene, in line with its activity in clinical samples as mentioned above. Up-regulation of SOX1 inhibited the growth of CC cells both in vitro and in vivo by impeding the transcriptional activity of T cell factor in the Wnt/β-catenin signaling pathway. It also promoted metastasis by up-regulating the cancer metastasis suppressor gene cadherin 1 (CDH1) and simultaneously down-regulating Snail2, the inhibitor of E-cadherin transcription. Future researches should focus on exploring how SOX1 influence cell apoptosis and treatment efficacy.

Intriguingly, although SOX9 and SOX14 were regarded as tumor suppressor genes with low expression level detected in patients’ samples due to methylation, these two SOX genes presented dual functions based on research on cell lines. As oncogenes, some researchers reported that overexpression of SOX9 in CC cell lines promoted cell proliferation by down-regulating the expression of PTEN, which prevents cells from growing too quickly. And overexpression of SOX14 were proved to boost cell proliferation and invasion by activating the Wnt/β-catenin signaling pathway along with high level of β-catenin. In contrast, as tumor suppressor genes, up-regulation of SOX9 or SOX14 could block the cell cycle transition by activating the expression of p21WAF1/CIP1 and p53, resulting in suppression of cell growth and tumor formation in vitro. Overexpression of SOX14 also induced apoptosis by promoting the expression of Bax and cleaved-poly ADP-ribose polymerase. These interesting results indicated the need for more thorough investigations to compare and combine the effects of different experimental conditions on outcomes.

**Effects of SOX genes on treatment efficacy in CC**

In terms of treatment, SOX2 and SOX4 are considered as oncogenes accordance with their roles in the progression of CC. Superficially, expression level of SOX2 was higher in tissues from patients with radiation-resistance compared with those with radiation-sensitivity, suggesting that SOX2 was a biomarker of unfavorable therapeutic reactivity. Overexpression of SOX genes, such as SOX4, in Caski cell lines also decreased the treatment efficacy of cisplatin by up-regulating the drug efflux transporter gene ABCG2. And more investigations are required to explore how SOX genes influence treatment efficacy in patients with CC. In addition to SOX2 and SOX4, one study implied that high expression of SOX9 in CC cell lines enhanced cell resistance to cisplatin through combining with the promoter region of miR-130a and down-regulating expression of copper transporter protein 1 (CTR1), which is a significant factor affecting the activity of cisplatin. However, this result was in contrast to the action of SOX9 in the progression of CC, and the function of SOX9 in the treatment of CC still needs clarification.

In conclusion, SOX genes play significant roles in CC, and SOX members may act as promising biomarkers or therapeutic targets in clinical practice in the near future.

**SOX genes in EC**

EC, with an estimated 63,230 new cases and approximately 11,350 deaths, was regarded as the most common female reproductive system malignancy in the United States in 2018. Despite extensive research focusing on exploring the genetic and epigenetic characteristics of EC, its pathogenesis and progression remain unclear. Recently, some studies indicated the important roles for SOX genes in regulating the progression of EC (Figure 6) and in indicating clinical features and treatment efficacy of patients with EC (Table 1).
Low expression level of tumor suppressor SOX genes, such as SOX1, SOX7, SOX9, and SOX17, due to methylation and other mechanisms, could be considered as novel prognostic biomarkers of EC. For example, low expression level of SOX1, SOX7, and SOX17 was shown to be potential biomarkers for detecting EC masked by atypical hyperplasia, and indicated advanced stage, higher grade, and shorter RFS.\(^{19,21,56,57,60}\) Additionally, high expression level of SOX17 in tissues indicated increased toxicity of cisplatin and high therapeutic sensitivity of patients.\(^{61}\) However, SOX9 expression showed a significant stepwise increase from normal tissues through grade 1 to grade 2/3 cancer tissues, probably due to a hidden feedback system.\(^{59}\) This study suggested that detecting the detailed mechanisms of SOX9 will provide valuable information.

Interestingly, SOX2 is identified as a bi-functional gene in EC, as in OC and CC. Pityński et al.\(^ {15}\) analyzed expression level of SOX2 in samples from EC patients and found higher expression level of it in high-grade (G3) compared with moderate-grade (G2) and low-grade (G1) of EC. High expression level of SOX2 in tissues was also associated with poorer outcomes of patients with advanced-stage EC.\(^ {11}\) In contrast, Wong et al.\(^ {58}\) proposed that SOX2 was a tumor suppressor gene inhibiting the progression of EC, and low level of it was identified as an indicator of type II serous, clear cell adenocarcinoma as well as shorter survival. These phenomena suggested that further exploration of the molecular mechanisms of SOX2 should be carried out in relation to clinical management of EC.

**SOX genes regulate progression of EC**

In relation to the progression of EC, researchers regulated the
Effects of SOX genes on treatment efficacy in EC

In relation to treatment of EC, only SOX17 was demonstrated an association with the chemosensitivity of EC cells to cisplatin. Zhang et al. overexpressed SOX17 in HEC-1B cells and found that cells with elevated expression of SOX17 had higher sensitivity to cisplatin, lower cell viability, and higher cell apoptosis rate when treated with cisplatin. These results suggest that SOX17 may be a promising target for gene treatment of EC.

Similarities of SOX genes in GC and prospective challenges

In this review, we classified SOX genes and emphasized their critical roles as regulators in the progression of GCs. And SOX genes were also considered as biomarkers of clinical features, including FIGO stage, histological grade, treatment efficacy, and prognosis of patients with GCs. SOX genes owned the potential to assist gynecologists in making precise clinical decisions. There were some similarities in SOX genes among GCs. 1) Methylation analysis of the tumor suppressor SOXI gene in patients’ samples was regarded as a novel method for early screening and as a biomarker of clinical features. 2) Expression level of the dual-acting SOX2 gene revealed low level in some cases and high level in other cases, indicating different prognostic values in different patients, and highlighting the need for more research to clarify its role.
3) At a cellular level, transduction of the oncogene SOX4 in cell lines promoted the progression of GCs by enhancing cell proliferation and metastasis. 4) SOX7 and SOX17, both belong to subgroup F SOX genes, acted as tumor suppressor genes which were associated with decreased rate of progression. Given that SOX3 belongs to subgroup B with SOX1 and SOX2, meanwhile, SOX18 belongs to subgroup F with SOX7 and SOX17. Researchers could investigate the roles of SOX3 and SOX18 in GCs to see if they play similar roles to other members from the same subgroups.

The current review was limited to studies of the three most common GCs and certain histological subtypes, such as cervical squamous cell carcinoma, epithelial OC, and unexplained EC. Therefore, larger population-based studies of other kinds and subtypes of GCs, such as uterine myoma, uterine sarcoma, tumors of the fallopian tube, and vulvar squamous cell carcinoma, are warranted to further our understanding of the associations between SOX genes and the progression of GCs. Although studies to date only scratched the surface in terms of understanding the biological and clinical functions of SOX genes in GCs, these pre-clinical studies held promise and provided the basis for future studies aiming at elucidating the detailed functions of these genes.

**Acknowledgments**

This work was supported by grants from the National Natural Science Foundation of China (Grant No. 81572568 and 81272863).

**Conflicts of interest statement**

No potential conflicts of interest are disclosed.

**References**


