

Expression of sex-determining region Y-box protein 2 in breast cancer and its clinical significance

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ABSTRACT

أن منطقة تحديد الجنس البروتين Y-2 (SOX2) هو عامل النسخ أنيمبريو الموجود على الكروموسوم 3q26.3-Q27. كما أنه يلعب دوراً هاماً في صيانة التمايز والتجديد الذاتي للخلايا الجذعية متعددة القدرات. وقد أظهرت الدراسات أن SOX2 يرتبط مع سرطانات متعددة و زيادة تعابير العديد من المظاهر المختلفة لسرطان الثدي. لدراسة العلاقة بين SOX2 والمعلومات السريرية الباثولوجية من مرضى سرطان الثدي، وجدنا أن التعبير عن SOX2 يرتبط ارتباطاً وثيقاً بالزيادة في حجم الورم، والصف النسيجي، والعقدة الليمفاوية، والانبثاث، وشدة الغزو. لذلك، الدراسات عن دور SOX2 في سرطان الثدي قد توفر المؤشرات الحيوية الفعالة والأهداف العلاجية المحتملة لتشخيص وعلاج سرطان الثدي. ستناقش هذه المقالة دور SOX2 في سرطان الثدي، بما في ذلك حدوثه، والغزو، والانبثاث، والتشخيص، والعلاج، والانتكاس، والمقاومة، والتشخيص.

Sex-determining region Y-box protein 2 (SOX2) is an embryo transcription factor located on chromosome 3q26.3-q27. It plays an important role in the maintenance of differentiation and self-renewal of pluripotent stem cells. Studies have shown that SOX2 is associated with multiple cancers and is overexpressed in many different phenotypes of breast cancer. To study the relationship between SOX2 and clinicopathological parameters of breast cancer patients, we found that the expression of SOX2 was closely related to the increase in tumor size, histological grade, lymph node metastasis, and high invasiveness. Therefore, studies on the role of SOX2 in breast cancer may provide effective biomarkers and potential therapeutic targets for the diagnosis and treatment of breast cancer. This article will discuss the role of SOX2 in breast cancer, including its occurrence, invasion and metastasis, diagnosis and treatment, relapse, resistance, and prognosis.

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Breast cancer is the leading cause of cancer death in women worldwide, and it is a kind of malignant tumor that has been extensively studied via genomic research. At present, the treatment of breast cancer includes surgical treatment, cytotoxic drug therapy, hormone replacement therapy, targeted therapy, immunotherapy, and radiotherapy. However, the treatment effect is not always ideal. Although some progress has been made in the diagnosis and treatment of breast cancer, the lack of effective blood markers that can be used for detection of breast cancer greatly restricts further improvement in the diagnosis and treatment of breast cancer. Adorno-Cruz et al¹ suggested that cancer stem cells (CSCs) are the source of recurrence and metastasis of many tumors, including breast cancer. Cancer stem cells are capable of self-renewal, unlimited proliferation, and multi-differentiation, similar to embryonic stem cells. They may form a tumor cell population with different phenotypes through abnormal differentiation. New research suggested that CSCs may be responsible for the development and metastasis of tumors.^{2,3} Transcription factor SOX2 (sex-determining region Y-box protein 2) is considered a marker of tumor stem cells because of its ability to maintain the differentiation potential and self-renewal capacity of embryonic stem cells and its potential role in cancer development. Research suggested that SOX2 is highly expressed in glioblastoma,⁴ lung cancer,⁵ breast cancer,⁶ gastric cancer,⁷ pancreatic cancer,⁸ colon cancer,⁹ prostate cancer,¹⁰ oral squamous cell carcinoma,¹¹ head and neck squamous cell carcinoma,¹² and squamous cell carcinoma of different organs.¹³ Its expression level is one of the markers of prognosis of a variety of tumors, and detection of SOX2 in the early stages of disease might be critical for early disease diagnosis and accurate

prognosis.¹⁴ The role of SOX2 in maintaining the self-renewal capacity of CSCs in breast cancer has been confirmed.¹⁵ Therefore, exploring the role of SOX2 in breast cancer may provide effective biomarkers for the diagnosis and treatment of breast cancer. This paper presents the role of SOX2 in breast cancer to provide effective biomarkers for the diagnosis and treatment of breast cancer, as well as a basis for further research on SOX2 and breast cancer.

1. Summary of SOX2. Sex-determining region Y-box protein 2 is an embryonic transcription factor located on chromosome 3q26.3-q27. Its encoded expression product, SOX2 protein, is constituted by 317 amino acids, and its molecular weight is 34.3 kDa.¹⁶ It plays an important role in the maintenance of the differentiation potential and self-renewal capacity of embryonic stem cells. Sex-determining region Y-box protein 2 has 3 main domains: the N-terminal domain, the high mobility group domain, and the transactivation domain.¹⁷ An increasing number of studies have shown that SOX2 is related to a variety of tumors. Silencing SOX2 can induce the transcription of p21Cip1 and p27Kip1, leading to cell cycle arrest and cell growth inhibition. Silencing the expression of SOX2 in lung squamous cell carcinoma results in cell suppression by up regulating the tumor suppressor BMP4.¹⁸ Knockdown of SOX2 in glioblastomas in immunodeficient mice reduces tumor cell proliferation and lost tumorigenicity.¹⁹ Sex-determining region Y-box protein 2 promotes cell proliferation in breast, prostate, and cervical cancers and involves the escape of apoptotic signals in prostate, gastric, and non-small cell lung cancer. These studies all suggested that SOX2 plays an important role in inhibiting cell apoptosis and promoting cell proliferation. Sex-determining region Y-box protein 2 mediates the aggressive and migratory phenotypes by activating MMP3, MMP2, and PI3K/AKT/mTOR,²⁰ which indicates that SOX2 also plays a role in tumor invasion and metastasis. Results suggest

that PI3K/Akt may play an important role in the expression of SOX2.

2. Sex-determining region Y-box protein 2 and breast cancer. The effect of SOX2 in breast cancer has gradually been identified with increasing study. Sex-determining region Y-box protein 2 is overexpressed in a variety of different phenotypes of breast cancer. In breast cancer, SOX2 has been found in sporadic basaloid carcinomas and in several early postmenopausal cancers.²¹ Lengerke et al²² evaluated SOX2 expression in primary tumors of 95 postmenopausal breast cancer patients, and they reported its association with various early postmenopausal breast cancer and lymph node metastases. Rodriguez-Pinilla et al²¹ showed high expression of SOX2 in basal-like breast cancer based on statistics. When they investigated the association of SOX2 with sporadic basal-like breast tumors, 43.3% showed SOX2 immunoreactivity, whereas SOX2 expression was only found in 13.3% of HER2-positive tumors and 10.6% of luminal tumors by immunohistochemical analysis of 226 sporadic node-negative infiltrative breast cancers. They reported that SOX2 expression is directly related to tumor size, CK5/6, epidermal growth factor receptor, and vimentin expression, and ER and PR expression decrease when SOX2 expression is upregulated. Zheng et al²³ reported that SOX2 expression is closely related to tumor size, histological grade, lymph node metastasis, and high invasive triple negative phenotype by analyzing the relationship between SOX2 and clinical pathological parameters of patients with breast cancer. They believed that the detection of SOX2 expression might have great value in determining the diagnosis and prognosis of patients with breast cancer. Huang et al²⁴ found that SOX2 is associated with the expression status of tumor-negative hormone receptor, high proliferative index, and neuroendocrine marker expression; moreover, it plays a role in poorly differentiated tumors. Sex-determining region Y-box protein 2 has different prognostic significance in 5 kinds of breast cancer: basal-like, luminal A and B, HER2+, and normal breast-like carcinomas.²⁵

2.1 SOX2 and the development of breast cancer

(*Figure 1*). To study the role of SOX2 in the development of breast tumors, Chen et al²⁶ found that overexpression of SOX2 increases the population of S + G2/M phase cells and decreases the G0/ G1 phase cell population. They used functional acquisition experiments and loss of function experiments to examine the effect of SOX2 overexpression or low expression on cell cycle regulation in breast cancer cells via retroviral-mediated overexpression and low expression strategies. Similarly,

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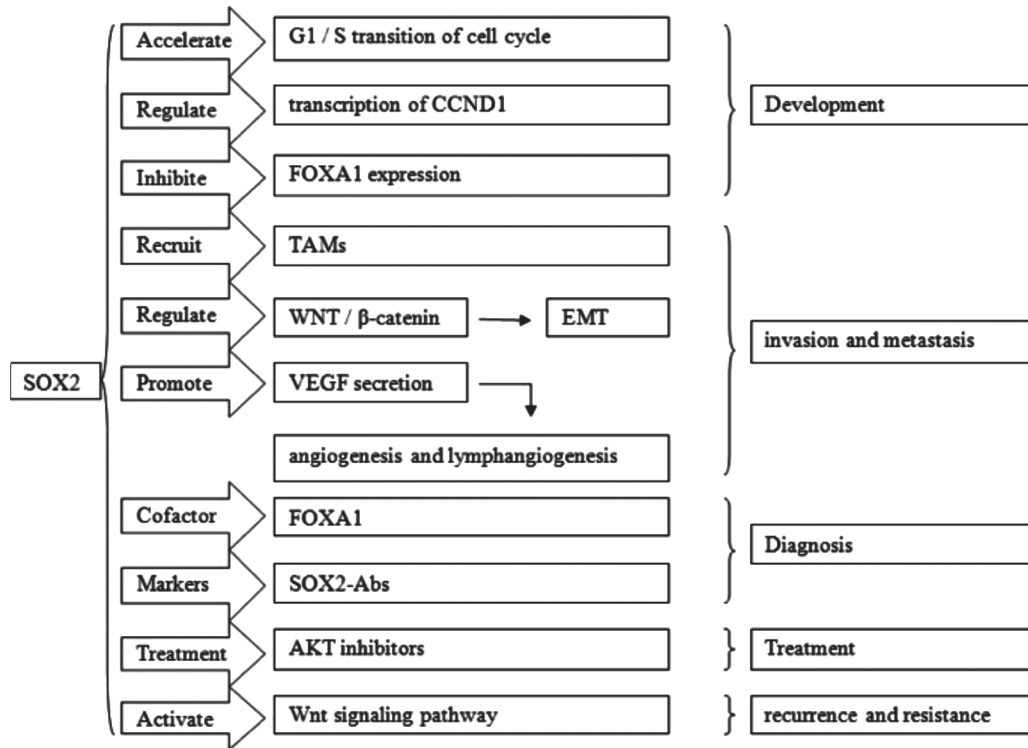


Figure 1 - Specific mechanism of SOX2 in the development, invasion and metastasis, diagnosis and treatment, recurrence, and resistance of breast cancer.

using siRNA to decrease the expression of SOX2 results in cell arrest at the G0/ G1 phase. This finding indicates that the accumulation of G0/G1 cells is specifically correlated with the loss of the SOX2 gene and SOX2 protein. Thus, SOX2 might promote cell proliferation and tumorigenesis of breast cancer by accelerating the G1/S transition of cell cycle, and SOX2 and β-catenin both regulate the transcription of cyclin D1 (CCND1) in breast cancer. Cyclin D1 may also play a certain role in the development of the disease. However, the specific mechanisms still need to be further explored.

2.2 SOX2 with the invasion and metastasis of breast cancer. The recruitment of tumor-associated macrophages promotes the metastasis of breast cancer. Sex-determining region Y-box protein 2-positive breast cancer cells can regulate the secretion of many cytokines such as MIP-1a/ CCL3 and ICAM. It also regulates the activation of the NF-κB and STAT3 signaling pathway to play an important role in the recruitment of tumor-associated macrophages.²⁷ Li et al²⁸ found that SOX2 promotes epithelial–mesenchymal transition to promote breast cancer metastasis by regulating the WNT/ β-catenin signaling pathway in breast cancer

cells. Lengerke et al²² reported similar associations in a meta analysis of the relationship between SOX2 and clinicopathological parameters in patients with breast cancer. Increasing SOX2 expression in early breast cancer might promote tumor metastasis. Although there is no conclusive evidence that SOX2 is associated with lymph node metastasis, recent studies have demonstrated that SOX2-positive patients present significantly higher lymph node metastasis rates than those with SOX2-negative disease.²⁹ Michifuri et al³⁰ stated that SOX2 is essential for invasion and proliferation of breast cancer, and SOX2-positive cancer cells have a higher ability and probability to transfer to the lymph nodes than SOX2-negative cancer cells; in addition, down regulation of SOX2 expression significantly reduces angiogenesis and lymphangiogenesis of breast cancer. Chinese studies have shown that SOX2 might promote angiogenesis and lymphangiogenesis by promoting vascular endothelial growth factor A secretion to affect tumor lymph node metastasis.³¹ These studies suggested that SOX2 plays a role in breast cancer metastasis through a variety of mechanisms. Sex-determining region Y-box protein 2 indicates poor prognosis, so it is a viable marker for prognosis in patients with breast cancer.

At present, the problems of SOX2 and invasive breast cancer remain controversial. Fang et al³² found that downregulation of SOX2 expression significantly increases invasiveness of breast cancer such as MCF7 and ZR751 in a study on the relationship between SOX2 and breast cancer cell invasion. Sex-determining region Y-box protein 2 inhibits the invasiveness of breast cancer cells through a mechanism that is dependent on Twist-1 and the transcriptional activity of SOX2. The latest Chinese studies revealed that SOX2-positive cells suggest high tumor invasion and lead to tumor lymph node metastasis by promoting blood vessels and lymphangiogenesis. Experimental results show that SOX2 plays a certain role in angiogenesis.³³ The relationship between SOX2 and invasive ability of breast cancer cells needs further confirmation.

2.3 SOX2 with the diagnosis and treatment of breast cancer. Li et al³⁴ detected the regulation effect of SOX2 in the expression of FOXA1 by reverse transcription polymerase chain reaction (RT-PCR) and Western blot. Their results revealed that SOX2 acts as a negative upstream regulator of the FOXA1 (Forkhead Box A1) gene and inhibits FOXA1 expression. They also found significantly higher tumor incidence because SOX2 and FOXA1 were both expressed abnormally compared with that in which only a single gene was expressed abnormally in invasive breast cancer. Immunohistochemical staining further revealed that SOX2 and FOXA1 demonstrates complementation in the diagnosis of human breast cancer. Therefore, SOX2 and FOXA1 can be used as effective markers for the diagnosis of breast cancer. The use of FOXA1, as a cofactor of SOX2, can improve the diagnostic efficiency of breast cancer.

In addition, studies found that SOX2-related serum, SOX2 autoantibody (SOX2-Abs), can be used as a serum marker of breast cancer. Sex-determining region Y-box protein 2-Abs was first reported in 2012. It is related to high tumor grade and positive lymph node status in breast cancer patients. Sun et al³⁵ determined the presence of SOX2-Ab in 282 breast cancer patients, and they discovered it in 78 patients with benign breast disease and 194 healthy women via indirect enzyme-linked immunosorbent assay. SOX2-Abs was generally found in breast cancer patients (18.4%) than among healthy women (2.6%) and patients with benign breast disease (6.4%). Comparing the sensitivity and specificity of SOX2Ab and serum CEA, TPS, CA125, and CA153 in patients with benign and malignant breast cancer proved that the sensitivity of the SOX2-Ab assay (18.4%) was significantly higher than that of CEA, CA125, and CA153 (all <5%), and its specificity

was superior to that of the TPS assay. Furthermore, SOX2-Abs measurement was more effective than serum CEA, TPS, CA 125, and CA 153 determination upon comparing the distinction between benign and malignant breast disease. Therefore, SOX2-Abs measurements were more powerful diagnostic tools than these clinically acceptable diagnostic markers. This study was the first report of the presence of SOX2-Abs, and its potential clinical applications may provide a noninvasive and simple serological marker for the diagnosis and prognosis of breast cancer.

The high expression of SOX2 is related to the occurrence of breast cancer. The inhibition of high SOX2 expression may provide a novel idea for the treatment of breast cancer. Schaefer et al³⁶ found that Ser/Thr-kinase AKT is an upstream regulator of SOX2 protein, and ectopic expression of SOX2 enhances the ability of clones of tumor cells and restores the cellular tumorigenicity of cells that were inhibited by AKT inhibitors in vivo. AKT inhibitors can effectively inhibit the growth of tumor stem cells with SOX2 expression on which conventional chemotherapeutic agents usually act. Therefore, AKT inhibitors can inhibit the expression of SOX2 protein. Novel treatment methods for breast cancer may be obtained by studying effective AKT inhibitors. In a recent study, Liu et al³⁷ demonstrated that a new SOX2-mediated regulatory axis plays an important role in the proliferation, invasion, and metastasis of breast cancer cells, thereby providing a novel direction for the treatment of breast cancer.

2.4 SOX2 with the recurrence and resistance of breast cancer. Sex-determining region Y-box protein 2 is associated with early recurrence of breast cancer. Finicelli et al³⁸ observed the effect of SOX2 on early recurrence in patients with breast cancer via multivariate analysis. The risk of recurrence with SOX2-positive disease (82%) was higher than that in SOX2-negative disease (42%) in 117 breast cancer samples. Therefore, patients with SOX2-positive disease will relapse earlier than patients with SOX2-negative disease.

Drug resistance of breast cancer has always been a clinical problem. CSCs play a role in tumor radiotherapy and chemotherapy resistance. Tamoxifen, an estrogen receptor antagonist, is the basic therapy of patients with breast cancer. Piva et al³⁹ found that tamoxifen-resistant cells are enriched in tumor stem/progenitor cells, and they express high levels of stem cell marker SOX2. The sensitivity of tamoxifen is negatively related to the ectopic expression of SOX2. The tumor stem/progenitor cell population decreases and sensitivity to tamoxifen is restored if the expression of SOX2 gene is knocked out. Compared with patients who were successful with endocrine therapy, the SOX2

level was higher in primary tumors of patients with failure to endocrine therapy. Gene expression profiles showed that the Wnt signaling pathway is activated in SOX2-expressing cells. It can restore sensitivity of drug-resistant cells to tamoxifen through inhibition of Wnt signaling. These results indicate that the development of tamoxifen resistance is mediated by SOX2-dependent activation of Wnt signaling in cancer stem/progenitor cells.

In conclusion. The role of SOX2 in maintaining the self-renewal capacity of CSCs in breast cancer has been confirmed, and it is overexpressed in different phenotypes of breast cancer. The expression of SOX2 is closely related to the increase in tumor size, histological grade, lymph node metastasis, and high invasiveness. Therefore, studies on the role of SOX2 in breast cancer may provide effective biomarkers and potential therapeutic targets for the diagnosis and treatment of early-stage breast cancer.

With the gradual recognition of the role of SOX2 in tumors, the specific mechanisms of SOX2 in tumor occurrence, invasion and metastasis, diagnosis and treatment, relapse, resistance, and prognosis are becoming research hotspots. Breast cancer remains the leading cause of women cancer death in the world. The global incidence of breast cancer are more than 20% and mortality 10%. It is a kind of malignant tumor with extensive genomics research. The recurrence, metastasis, drug resistance, and other issues of breast cancer seriously affect the quality of life of patients. Therefore, studies on the mechanism of the development of SOX2 in human breast cancer are crucial. Further research on SOX2 may determine the specific mechanism of SOX2 in breast cancer to provide novel techniques for the detection, diagnosis, treatment, and prognosis of breast cancer. The intervention of the mechanism of SOX2 action will provide a new therapeutic target for the treatment of breast cancer, as well as an appropriate detection index for the prognosis of breast cancer.

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