

## Sox2 gene expression as a gene to change the aggressive behavior of cancerous cells in patients with gastric cancer

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### Abstract

**Introduction:** Epithelial–mesenchymal transition (EMT) phenomenon in cancer cells is one of the most sensitive stages in metastasis. In addition, it has been shown that many molecular factors and cellular signals, including the Sox2 gene, are involved in arising EMT, which together cause EMT. The phenomenon of EMT in cancer cells is one of the most sensitive stages in metastasis. Expression of Sox2 gene as an effective gene in altering aggressive behavior of cancer cells has not been studied in patients with gastric cancer.

**Materials and methods:** RNA was extracted from 50 tumoral and 50 normal samples from patients with gastric cancer. Then cDNA synthesis was performed on the extracted RNAs. Primers for the target genes were designed and synthesized. Quantitative real-time PCR was performed to determine the relative expression of the studied genes and the relationship between the clinic pathologic parameters and the amount of RNA was analyzed by Pearson correlation coefficient analysis.

**Results:** mRNA expression of Sox2 in tumor tissue was significantly higher than that in healthy adjacent tumor tissue. The mRNA expression level of Sox2 in tumor tissue was significantly higher than that in healthy adjacent tumor tissue. Also, mRNA levels of Sox2 in tumor tissue indicated a direct and significant correlation with tumor staging (TNM stages) ( $\tau = 0.329$ ,  $P = 0.02$ ). In addition, there was no significant correlation between mRNA levels of Sox2 in tumor tissue and tumor size ( $\tau = 0.138$ ,  $P = 0.177$ ).

**Conclusion:** Assessment of Sox2 gene expression in the study of genes involved in EMT process in gastric cancer patients and its relation to pathologic, clinical and metastatic findings in gastric cancer is an effective method in the diagnosis of gastric cancer.

**Keywords:** Gastric cancer, Sox2, Gene expression

### Introduction

Cancer is a serious global health issue and is the second leading cause of death after cardiovascular disease (1). Gastric cancer is the fourth leading cause of human malignant disease and the second leading cause of

cancer death, such that close to 800000 people die every year because of gastric cancer (2,3). Despite the great efforts and advances made in understanding the molecular factors involved in the treatment

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and diagnosis, there is no definitive cure for this disease. Recent therapies have greatly improved patients' quality of life and treatment expectations, however the impact of these treatments and predicting the course of the disease progress largely depend on the stage at which the tumor is diagnosed (4).

An important feature of this disease is the absence of clinical symptoms in the early stages of development that will progress and reach an advanced stage. This feature has caused that 20 to 30 percent of patients have a survival rate of about 5 years after treatment, and 61 percent of those diagnosed with early-stage disease have a chance of survival (5).

Although endoscopic procedures and microscopic tests are reliable techniques for early detection of gastric cancer, these methods are invasive and inappropriate for routine screening. The advanced stages of cancer, usually associated with distant metastasis, are uncontrollable. Metastasis is the result of changes in the body's molecular system and tumor mass. Epithelial–mesenchymal transition (EMT) is one of these changes. EMT or epithelial-mesenchymal morphology transition in cancer cells is one of the most sensitive stages in metastasis (6). In addition, EMT has been found to be the major stage in morphogenesis during embryonic development. Evidence suggests that EMT promotes metastasis, drug resistance, and tumor recurrence (7). Many molecular factors and cellular signals are involved in the development of EMT. In EMT, cancer cells move to blood vessels and invade distant tissues (8). When cancer cells leave the blood or lymph vessels and enter other tissues of the body, to survive and implant in new tissue (metastasis) they need to reconnect with each other and with new tissue cells. Thus, cancer cells re-express the proteins responsible for cellular attachment on their surface, thus leaving the mesenchymal state and transform to the shape of epithelial cells. MET has been recognized as an important step during

embryonic development. Several factors are involved in these behavioral and aggressive changes in cancer cells. For mesenchymal changes, the intercellular junctions will first be changed, followed by the altered cytoskeleton. For mesenchymal changes, the intercellular junctions and cytoskeleton will have altered, and after separation of the cells, the cell will acquire the mesenchymal phenotype. Consequently, after extracellular matrix changes which mediated by enzymes that degrade ECM, the potential for cellular motility and invasion increases (9). Once the cell enters the MET stage it can no longer invade the tissue but can grow and cause malignant tumors. Understanding EMT and identifying the role of its contributing factors in regard with clinical changes is very important. Today's, genes that are involved in the control and self-regeneration of stem cells have been introduced as a new class of cancer molecular markers whose uncontrolled expression is of great importance in the process of cell cancer (10). The *sox2* gene is one of these genes and is an important gene in the development of self-regeneration capacity such that it activates stem cells and inhibits the differentiation initiator genes, which in turn maintaining the self-regeneration of the stem cells (11-15).

*Sox2* is one of the most important transcription factors that involved in the regulation of embryonic stem cell self-regeneration. This transcription factor is a member of the HMG protein family and plays an important role in transcription of many genes. Researchers at Massachusetts General Hospital (MGH) and the Harvard Stem Cell Institute (HSCI) also confirmed that *Sox2*-expressing cells in the stomach, testis, cervix and other structures are true adult stem cells that can transform to all types of mature stem cells (16 - 22). It seems that *Sox2* is the only transcription factor in stem cells that is expressed at all stages of development, whether embryonic, embryonic or adult.

## Material and method

**Samples:** A total of 50 gastric cancer tumoral biopsy specimens and 50 adjacent normal tissue specimens were selected for the study. The specimens were collected from patients with gastric cancer who referred to Cancer Institute of Iran between 2007 and 2016. The frozen tissue specimens of these patients were pre-selected by a pathologist and kept at the Iran tumor Bank.

**RNA Extraction:** RNA was extracted from all samples using the Qiagen Rneasy Mini Kit according to the manufacture instructions. Then, the purification and quantitative evaluation of RNA were determined by the Optical Density (OD) method and the quality of the RNAs was electrophoresed on agarose gel.

**cDNA synthesis:** Possible genomic DNA residues in RNA-containing tubes were removed by exposing the tubes to Dnase enzyme (RNase-Free DNase Set, Qiagen) for 5 min at 42 °C And then by rapid placement of the samples on ice. mRNAs (500 ng of each sample) using a commercial kit (Quantitect Reverse Transcription kit) was transformed into cDNA according to the manufacture instructions. The operating conditions of the Thermal cycler involved a temperature cycle of 42 °C for 15 minutes, a stop step at 95 °C for 5 minutes, the addition of 91 µl of free-nuclease water to the microtubes, and mixing and rapid placement on zinc. Reverse transcription reaction solution (cDNA) can be stored at 20 °C. If qPCR (Real-time PCR) was performed immediately, the solutions would remain on ice until the next step, i.e. real-time PCR performed.

**Quantitative Real time PCR (qRT-PCR):** cDNAs that produced in the previous step were amplified with a Mastermix mixture (RT2 SYBR Green ROX qPCR Mastermixes, Qiagen).

The mixture of the cDNA reaction and the corresponding primers were already added to each Real-time PCR plate and then 25 µl of the Mastermix mixture was added to

each well. The sequence of the primers used were 5'-ACCAGCTCGCAGACCTACA-3 (SOX1-forward) and 5'-GGACTTGACCACCGAACC-3' (SOX2-reverses).

The tubes were centrifuged at 1000 g for one minute at room temperature. The contents of the tubes were then amplified in 40 temperature cycles of Real-time PCR (Bioneer Exicycler TM96) at 95 °C for 10 minutes, 95 °C for 15 seconds, 55 °C for 30-40 seconds, and 72 °C for 30 seconds. The cycle threshold (CT) was determined using device software. The amplification curve was then plotted on a Y axis using a logarithmic scale ranging from 0.01 to 10. The mRNA was identified by analyzing the main peak of melting curve related to amplification. Serial dilutions of standard mRNA were prepared and amplification was performed to determine the changes in expression of each mRNA in the samples. Then, a standard curve was drawn for each primer based on CT scans. mRNA of 2Mβ control gene was used to normalize the mRNA levels among the samples.

## Statistical analysis

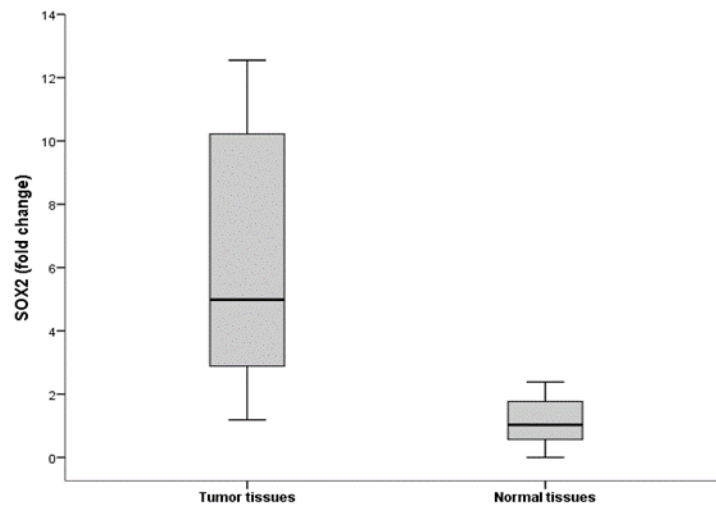
The correlation between the clinic pathological parameters and the amount of RNA was analyzed by Pearson correlation coefficient analysis. Paired t-test was used to compare the amount of RNA between tumor samples and adjacent healthy tissue. Kendall's tau coefficient was used to investigate the association between the amount of RNA and tumor staging (TNM staging). Significance level was considered as  $P < 0.05$ .

## Results

The results indicated that the association between mRNA levels of Sox2 gene in tumor tissue and tumor stage (TNM stages) in patients with gastric cancer are significant and direct. Also, there was no significant relationship between mRNA levels of Sox2 in tumor tissue and the tumor size or diameter ( $\tau = 0.138$ ,  $P = 0.177$ ).

The figure 1 indicated mRNA levels of Sox2 gene in tumor tissue and adjacent normal tissue in patients with gastric cancer. mRNA

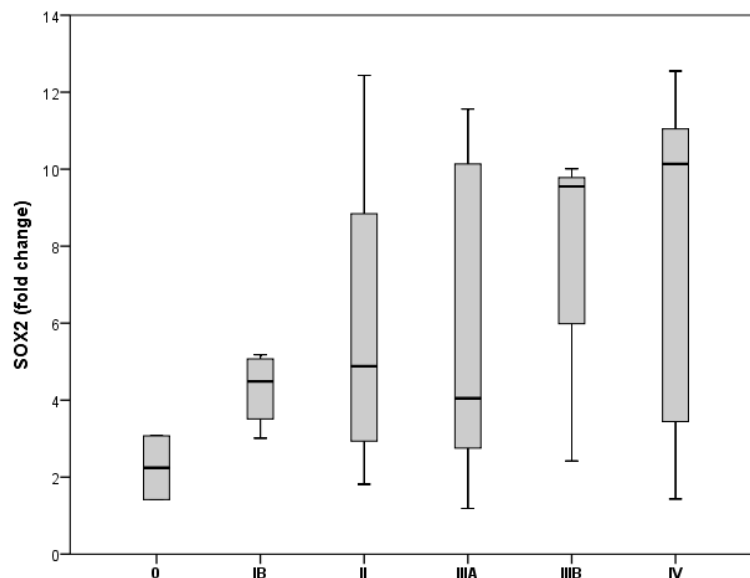
expression levels of Sox2 gene in tumor tissue is significantly higher than that in normal tissue adjacent to the tumor ( $P=0.0001$ ).



**Figure1.** mRNA levels of Sox2 gene in tumor tissue and adjacent normal tissue in patients with gastric cancer.

Also, mRNA levels of Sox2 gene in tumor tissue and has a direct correlation with tumor staging (TNM stages) indicated ( $\tau = 0.329$ ,  $P = 0.02$ ) (Figure 2). In tumor tissue, mRNA levels

of Sox2 indicated a significant and direct relationship with tumor staging (TNM stages) ( $\tau = 0.329$ ,  $P = 0.02$ ).



**Figure 2.** The association between the quantities of Sox2 gene mRNA in tumor tissue and tumor stage (TNM stages 0- IV) in patients with gastric cancer.

## Discussion

Based on these evidences, it can be concluded that the expression level of Sox2 gene is increased by gastric cancer tumor tissue, as well as the relationship between Sox2 gene mRNA levels in tumor tissue and TNM staging in gastric cancer patients is direct and significant. Clinical evidence and metastasis in gastric cancer process is an effective method in the diagnosis of gastric cancer. Colon cancer is the most common cancer in half of the country which is associated with digestive system and gastrointestinal tract cancers, and is the second most common cause of cancer death. Since most cancers are occur in older people and Iran's population is relatively young, it is expected in the near future that the death rate from this disease rapidly increased in the country. Therefore, preventive measures against this deadly disease and cancer control program are necessary in the country (12). An advanced stage of cancer with distant metastases is usually uncontrollable and therefore epithelial–mesenchymal transition (EMT) phenomena in cancer cells is one of the critical stages of metastasis. The advanced stages of cancer, usually associated with distant metastasis, are uncontrollable. The phenomenon of epithelial to mesenchymal transition in cancer cells is one of the most sensitive stages in metastasis. In this phenomenon, cancer cells develop mesenchymal morphology and move to blood vessels and invade distant tissues. Several factors are involved in initiating changes in the aggressive behavior of cells. Understanding the role of these factors in changing the aggressive behavior of cancer cells, especially in clinical trials, can be an important step in understanding the mechanisms involved in metastasis and hence its control. Cancer cell metastasis from primary tumors is one of the leading causes of poor prognosis and disease-related death. Metastasis spread accounts for about 90% of all cancer-related deaths. Therefore, early detection of cancer cell metastasis is of

great benefit to the therapeutic intervention and timely management of the disease (10). Metastasis is the leading cause of death in cancer patients. As a result of tumor invasion and metastasis, cancer cells migrate away from their primary mass, which include multiple stages, such as intravasation, internal invasion, migration to other organs, external invasion and micro-metastasis, and colony formation (8). Epithelial–mesenchymal transition phenomena is a chain of events that begins with changes in cell to cell adhesion and cytoskeleton. This phenomenon can lead to cellular changes of the extracellular matrix (ECM) and release of epithelial cells from peripheral tissue (12).

EMT is a complex process in which polar epithelial cells are changed to pseudo-fibroblastic non-polar change cells, a phenomenon that is the root of morphogenesis (morphogenesis) of tissue and organogenesis in the embryo as well as reconstruction and repair of tissues in adults. In addition, improper EMT reactivation play a significant role in the pathology of fibrotic diseases and cancer, including gastric cancer. Mammalian epithelial cells (MECs) have a squamous appearance and are interconnected through multiple cell-cell functions, including desmosomes, adherents, cleft and tight junctions.

Generally, these connecting structures give the MECs a polar characteristic. In contrast, Mesenchymal cells lack cell-to-cell adhesion complexes, leading to non-polar morphology and consequently increasing their migratory activity through the ECM (13).

EMT is divided into three categories: (a) embryonic and growth EMT which known as type 1 EMT; (b) fibrotic and tissue repair which known as type 2 EMT; and (iii) cancer and metastatic progression which known as type 3 EMT. Genetic changes as a result of cancer cells facilitate induction of type 3 EMTs. These EMTs produce cells with invasive properties that enable them to move in the bloodstream and to be

systematically transmitted to other organs (14). One of the most important mechanisms involved in the metastasis process is angiogenesis. Tumor cells require more oxygen than normal tissue and are therefore prone to hypoxia and angiogenesis. It has been suggested as a transcription factor in cellular self-regeneration in the embryonic period. Increased expression of this factor appears to be related to the induction of stem cell high potency (19). In a study by Bornschein J and colleagues on 48 gastric cancer patients, it has been found that Sox2 gene expression was dramatically reduced in tumor tissue (16).

### Conclusion

Based on this evidence, it can be concluded that the expression level of Sox2 gene were significantly increased by gastric cancer tumor tissue, as well as the association between the Sox2 gene mRNA levels in tumor tissue and TNM stages in gastric cancer patients were significant. As a result,

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