

# SOX 2as A Prognostic Marker in Malignant Epithelial Ovarian Tumors

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## *Abstract*

*Background: Ovarian cancer (OC) is considered the most lethal cancer of the female reproductive system and ranked as the seventh most frequent cancer diagnosed worldwide and the eighth leading cause of cancer-related death among women worldwide. The most common type of Ovarian cancer is that originate from the ovarian surface epithelium "Epithelial ovarian cancer" (EOC) which accounts for over 90% of all ovarian malignancies and is the most lethal gynecologic malignancy. Sex determining region Y-box 2 (SRY)-box 2 (SOX2) is one of the main members of the Sox family which consists of at least 20 members that are divided into 8 groups (from A to H, based on their high mobility group (HMG) sequence identity in humans). SOX2 expression has been reported at both the RNA and protein levels for many tumors, data available from The Cancer Genome Atlas indicates that SOX2 mRNA is elevated in many cancers, relative to normal tissue. The level of SOX2 expression in a tumor represents a prognostic factor to determine the clinical outcome for a cancer patient. As SOX2 has a great role in cancer stemness so SOX2 expression in OC is an interesting issue for studying.*

*Key words: Epithelial ovarian cancer (EOC), Sex-Determining Region Y-box2 (SOX2).*

**Ovarian Cancer:**

The incidence of ovarian cancer (OC) varies across the world, the epidemiological diversity of ovarian cancer in different regions is attributed to the risk factors that account for the occurrence of ovarian cancer. The highest incidence rates are observed in developed parts of the world, including North America and Central and Eastern Europe, with rates generally exceeding 8 per 100,000. Rates are intermediate in South America (5.8 per 100,000), and lowest in Asia and Africa ( $\leq 3$  per 100,000) (1).

**Malignant ovarian tumors are classified into two broad categories (1):**

1. Epithelial ovarian cancer (EOC)
2. Non epithelial ovarian cancer (NEOC).

**Classification of Epithelial ovarian cancer (EOC)**

The most common type of Ovarian cancer is that originate from the ovarian surface epithelium "**Epithelial ovarian cancer**" (EOC) which accounts for over 90% of all ovarian malignancies and is the most lethal gynecologic malignancy (2).

The histotyping of EOC is very important, as different clinico-pathological and molecular features of EOC subtypes have different approaches in clinical management which relies on histology and the immunohistochemistry of tumor biopsies (3).

The **World Health Organization (WHO)** classification (2014) of EOC (3).

1. Serous carcinoma.
2. Mucinous carcinoma.
3. Endometrioid carcinoma.
4. Clear cell carcinoma.
5. Transitional cell carcinoma.
6. Undifferentiated types.
7. Unclassified tumors.
8. Mixed malignant Mullerian tumors (carcinosarcoma).

EOC can be divided into two types with differing prognosis (4).

**Pathology of EOC types****Low-grade serous carcinomas (LGSC):****Incidence:**

Accounting for < 5% of EOC. They are diagnosed at a younger median age. They are slow-growing tumors with a 10-year survival rate of 50% (5).

**Morphology:****Gross picture:**

LGSC tend to be solid but lack conspicuous areas of necrosis and hemorrhage.

**Microscopic picture:**

LGSC is characterized by low-grade nuclear atypia, mitotic activity (<12 mitoses/10 HPF), stratification, glandular complexity, branching papillary fronds, and stromal invasion (6).

Stromal invasion in low-grade serous carcinoma can show a number of patterns, which may be admixed in a single tumor and include:

- Single cells and small, compact, irregular nests of cells
- Micropapillae and complex papillary forms
- Inverted macropapillae, in which the neoplastic epithelium covers broad fibrovascular stromal stalks (this is the least common pattern)
- Cribriform
- Glandular or cystic
- Solid sheets with slit-like spaces.

In all of these patterns there is typically a clear space surrounding the neoplastic cells.

**Serous psammocarcinoma**, is a rare variant of low-grade serous carcinoma characterized by massive psammoma body formation, ciliated cells are uncommon in low-grade serous carcinoma.

The microinvasive foci typically appear as individual cells or clusters of cells with abundant eosinophilic cytoplasm “eosinophilic metaplastic cells” that show a low Ki67 labeling index. It should be noted that in 50% or more of low-grade serous carcinomas there is a coexisting borderline component, from which the low-grade serous carcinoma has arisen (7).

**Immunohistochemical profile:**

Immunohistochemical profile is similar to HGSC (e.g., positive for CK7, wilm’s tumor 1 WT1), but LGSC never demonstrate aberrant p53 expression and also associated with negative p16 expression (8).

Low grade serous ovarian carcinoma includes an increased expression of ER compared to the HGSC (5).

The MIB-1 (Ki67) expression is higher in the HGSC than the LGSC, its immunostaining pattern is focal and heterogenous in low grade tumours as compared to diffuse pattern in higher grade (9).

**Molecular morphology:**

Somatic mutations in KRAS and BRAF can be found in approximately half of cases of LGSC most probably patients not displaying RAS pathway mutations have a late stage of disease development and poor survival (10).

**High-grade serous carcinomas (HGSC):****Incidence:**

Accounting for 75% of all EOC, it is a very aggressive tumor responsible for 90% of ovarian cancer deaths and there are no currently precise criteria to detect it in the early stage (11).

**Morphology:****Gross picture:**

HGSC are typically solid and cystic with areas of hemorrhage and necrosis, the tumor tissue is soft and friable, and most cases are bilateral.

In many cases the fallopian tube is completely obliterated by the adnexal mass such that normal tube or ovarian tissue cannot be identified. In some cases the tumor can be seen to involve the fimbriated end of the fallopian tube as a polypoid growth. In other cases, there is more diffuse enlargement of the fallopian tube by intraluminal tumor (12).

The omentum can be diffusely involved and called "omental cake" or may show multiple discrete nodules of tumor. The peritoneal surfaces often are studded with tumor "implants" of metastatic carcinoma (13).

**Microscopic picture:**

HGSC show a variety of architectural growth patterns, two or more of these patterns frequently coexist in the same tumor. The most characteristic growth pattern is termed "papillary", papillae with well-formed lymphovascular cores are less common than tufting of highly stratified epithelium, with fenestrated appearance and slit-like spaces.

Psammoma bodies may be present. Solid and glandular architectural patterns are also common. The solid pattern consists of sheets of cells without gland formation. Focally, slit-like spaces may be seen. Glandular differentiation is relatively common in HGSC, with well-formed glandular spaces lined by cells with high-grade nuclear features that are identical to those seen in the solid or papillary areas. HGSC may show a microcystic pattern with small, rounded spaces within sheets of tumor cells (14).

Clear cell change as a focal finding in HGSC is relatively common and this can be designated "HGSC with clear cell change" but should not be diagnosed as mixed HGSC/clear cell carcinoma, a tumor type that is rarely if ever encountered in practice (3).

**Immunohistochemical profile:****1. P53:**

HGSC is characterized by high frequency of pathogenic TP53 mutations, p53 has been used as a surrogate marker for the presence of TP53 mutations in HGSC.

While the p53 overexpression has been shown to correlate with serous carcinoma, the absence of p53 staining does not entirely exclude serous carcinoma, as the p53 protein may be truncated due to frame-shift mutation leading to null p53 immunostaining,

furthermore, the aberrant p53 immunoreactivity has also been reported in up to 37% of high-grade endometrioid carcinoma (15).

## **2. The MIB-1 (Ki67)**

Proliferation index and the expression are higher in HGSC compared with LGSC. Ki-67 antigen is over expressed in malignant ovarian tumour compared to benign or borderline tumours of surface epithelial origin. Its expression can be used to guide the clinical management of ovarian carcinoma both as a prognostic tool. High Ki-67 expression correlates with higher Grade, poor differentiation, ascites, vascular invasion, tumour metastasis. The presence of residual disease after primary surgery and advanced FIGO stage (16).

## **3. Wilm's tumor 1 (WT 1) :**

More than 90% of tubal/ovarian HGSCs are WT1 positive. In the female genital tract, WT1 expression is usually used to distinguish serous ovarian carcinomas from other ovarian tumour types and its immunoexpression mostly correlate with prognosis in ovarian cancer (8).

## **4. P16**

**P16** is a cyclin-dependent kinase-IV inhibitor that mediates its action through inhibitory effect on the cell cycle, P16 is well expressed in serous ovarian cancer and its expression is generally higher in high-grade tumors. P16 exhibits diffuse 'block-type' immunoreactivity in approximately two-thirds of HGSCs, while most of the remainder exhibit focal immunoreactivity (17).

## **5. Paired-box gene 8 (PAX8)**

Is expressed in 85–90% of HGSC and is a widely used biomarker for HGSC (18).

## **6. The epithelial marker CK7**

Is positive in HGSC but CK20 is usually negative (13).

### **Molecular profile:**

HGSC is characterized by defects in cellular mechanisms that allow for high-fidelity repair of DNA double strand breaks. This leads to mutation-prone repair mechanisms and resultant aneuploidy/copy number abnormalities (14).

Genetic abnormalities occur rapidly within the dividing tumor cells, leading to dramatic intratumoral heterogeneity at both the genetic and morphologic level (19).

A minority of HGSCs have amplification of cyclin E1 (CCNE1), a molecular abnormality that is mutually exclusive of BRCA mutation and is associated with a worse prognosis/platinum resistance, compared to HGSCs (20).

CCNE amplification often coexists with AKT amplification, and treatment directed against cyclin-dependent kinase-2 (CDK2) and AKT, with small molecule kinase inhibitors, is a potential approach to targeted treatment of these platinum resistant HGSCs (21).

CDK12 is a tumor suppressor gene, its mutations impede catalytic activity and cells with catalytically inactive CDK12 have defect in homologous recombination (HR). Similarly, disabling CDK12 in ovarian cancer cells disrupts HR and also sensitizes cells to DNA cross-linking agents and poly ADP-ribose polymerase (PARP) inhibitors (22).

Mutations of KRAS proto-oncogene, GTPase (KRAS), B-Raf proto-oncogene, serine/threonine kinase (BRAF) or (erb-b2 receptor tyrosine kinase 2) ERBB2 are infrequent (23).

### **Mucinous carcinoma:**

#### **Incidence:**

Accounting for only 5-10% of EOC. Mucinous neoplasms generally occur in young women and are diagnosed at an early stage, with 83% being diagnosed at stage I and only 17% diagnosed at stage II or higher (24).

#### **Morphology:**

##### **Grossly:**

They are typically large, unilocular, or multilocular cysts filled with mucoid liquid that becomes gelatinous at room temperature. The mean size at diagnosis is 18 cm, but these tumors can be massive and fill the abdomen and pelvis (25).

##### **Microscopically:**

The diagnosis of invasive mucinous carcinoma is based on the presence of stromal invasion more than 5 mm in depth or more than 10 mm in area. In 2014, World Health Organization (WHO) guidelines proposed classifying the primary mucinous cancers in these two groups based on their growth patterns, calling them expansile- and infiltrative-type tumors (26).

**The expansile (confluent) type** which is composed of confluent glands and a papillary pattern without intervening normal ovarian parenchyma and has a very good prognosis (27).

**The infiltrative type** which consists of glands, nest, or individual cells that infiltrate the stroma; and it appears to be more clinically aggressive and a higher risk of extraovarian spread (24).

Differential diagnosis between primary mucinous carcinoma and mucinous metastasis from other organs: It relies on some clinical characteristics including bilaterality, surface involvement, signet ring cell presence, and lymph vascular invasion, which are more common in metastases and quite rare in primary mucinous carcinoma. The tumor size 10 cm with a coexistent borderline and Brenner or dermoid tumors are clinical characteristics suggestive of primary mucinous carcinoma (28).

It has also been reported that mucinous ovarian neoplasms associated with pseudomyxomaperitonei are now considered derived from appendiceal primary tumors (29).

**Immunohistochemical profile:**

In contrast to other types of ovarian epithelial tumor, PAX8 is often negative, as well as WT1, but show CK7, CK20 and CDX2 positivity (30).

**Molecular morphology:**

In contrast to serous carcinomas, *K*-ras mutations are identified in 43–65 % of MOCs (and these have been identified in benign and borderline areas as well as in mucinous carcinomas, supporting the sequential tumorigenesis process going from a low-grade malignant mucinous lesion to an mucinous carcinoma. Mutations in the p53 tumor suppressor gene are present in some cases. Human epidermal growth factor receptor 2, HER2 (ERBB2) gene amplification has been reported in approximately 20–30% of invasive mucinous ovarian cancers. Amplification of Her2 has been identified in 19 % of mucinous tumors, which may provide a rationale for directed therapy in these cancers. Mucinous carcinoma does not appear to be linked to BRCA gene mutations, as only about 2 % of ovarian cancers associated with BRCA mutations are of mucinous histology (31).

**Seromucinous tumors:**

**Morphology:** It is considered as a new category that was added to the new WHO classification in 2014. Referred to as an endocervical type (Müllerian) of mucinous ovarian carcinoma. They are composed of an admixture of architectural patterns and cell types, including serous, endocervical-type mucinous, endometrioid, and squamous cells showing predominant papillary architecture with lesser components of glandular, microglandular, and solid growth (32).

**Immunohistochemical profile:**

On immunohistochemistry, CK7, hormone receptors, CA125, CA19.9, and PAX8 are consistently positive, and some cases are positive for WT1 but CK20 and CDX2 are generally negative (33).

**Molecular morphology:**

Approximately 33% of all seromucinous-type tumors present with a mutation in the tumor-suppressor gene ARID1A; therefore, it can be considered as one of the responsible genetic factors (34).

**Clear cell Carcinomas:****Incidence:**

Clear cell carcinoma accounts for 10% of ovarian carcinomas, which is mostly diagnosed in younger patients than other EOC subtypes (median age of 57 years), and at an earlier stage (35).

**Morphology:**

**Grossly:**

These tumors tend to be spongy with cystic appearance, but it contains several mural nodules (6)

**Microscopically:**

These tumors may have tubulo-cystic, papillary, and solid-sheet patterns (36).

Stroma-rich variants of clear cell carcinoma, with a prominent adenofibromatous component, are also common. In contrast to serous carcinomas, the papillae of clear cell carcinoma have a branched pattern and lack epithelial stratification (36).

The presence of cells with clear cytoplasm and hobnailed cells is common giving this cancer its name. The clear cytoplasm is filled with glycogen deposits, and stains positively with periodic acid-Schiff stain (3).

**Immunohistochemical profile:**

The MIB-1 (Ki67): The survival rate of the patients with high Ki-67 antigen expression was significantly greater than for low Ki-67 antigen expression and suggested low proliferation activity may contribute to chemoresistance(9).

These tumors exhibit positive immunostaining for hepatocyte nuclear factor-1 $\beta$  (HNF1b) and napsin A and an absence of immunostaining for Wilms tumour 1 (WT1) and estrogen receptor (ER).

Kisspeptin and Insulin-like growth factor II mRNA-binding protein 3 (IMP3) are prognostic in clear cell ovarian carcinoma but not in other ovarian cancer subtypes (37).

**Molecular pathology:**

Ovarian clear cell carcinoma are generally p53 wild type and have a lower frequency of breast cancer 1 (BRCA1) and BRCA2 mutations. Tumors with AT-rich interaction domain 1A (ARID1A) tumor suppressor gene mutations also frequently harbor phosphatase and tensin homolog (PTEN) or PIK3CA mutations, suggesting their collaboration in clear cell carcinoma tumorigenesis. Inactivation of ARID1A alone is insufficient for tumor initiation; it requires additional genetic alterations such as PIK3CA to drive clear cell carcinoma tumorigenesis (38).

**Endometrioid carcinomas (EC):**

**Incidence:**

Ovarian endometrioid carcinomas represent approximately 10% of all ovarian carcinomas (39).

**Morphology:**

**Grossly:**

Endometrioid carcinoma may present as a cystic or solid mass. The content tends to be hemorrhagic rather than serous or mucinous. Visible papillary formations are usually absent or inconspicuous (6).

**Microscopically:**

They strongly resemble the appearance of the ordinary type of endometrial adenocarcinoma hence their name. Several characteristic histologic features are seen in endometrioid carcinoma, including squamous morules, mucinous differentiation, clear cell change, spindle morphology, and secretory change (40).

**Immunohistochemical profile:**

WT1 expression is typically absent in endometrioid carcinoma (although expressed in up to 10% of cases) (41).

**Molecular pathology:**

The most frequently mutated gene is AT-rich interaction domain 1A (ARID1A) tumor suppressor gene, ARID1A may be an early event of endometriosis progression to cancer. P53 and P16: Alternatively, the utilization of p53 along with p16 may increase the sensitivity of accurate identification of serous immunophenotype. However, rarely the high-grade endometrioid carcinoma may also show variable aberrant immunoreactivity for both p53 and p16. The utilization of sensitive immunohistochemical markers such as p53 and p16 combined with other more specific tumor markers can assist in more accurate differentiation of serous carcinoma from high-grade endometrioid carcinoma (42).

Mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit  $\alpha$  (PIK3CA)/ phosphatase and tensin homolog (PTEN) and Wnt/ $\beta$ -catenin signaling pathways are frequently detected, including catenin beta-1 (CTNNB1) (15–40%), PIK3CA (20%) and PTEN (14–21%). CTNNB1 mutation is associated with squamous differentiation, low-grade tumors, and good prognosis (3).

PIK3CA and PTEN mutations can occur simultaneously. 13–20% cases of endometrial carcinoma possess microsatellite instability, which usually manifest as the deletion expression of hMLH1 (human mutL homolog1) or hMSH2 (human mutS homolog2) protein. KRAS and BRAF mutations are rare, with an incidence of less than 7% (2).

**Transitional-cell (Brenner) carcinoma:****Incidence:**

Brenner tumors of the ovary represent approximately 2% of all primary surface epithelial ovarian tumors and malignant Brenner tumors are quite rare, comprising approximately 1% of Brenner tumors. Malignant Brenner tumors occur during the fifth to sixth decades (mean age 55 years) (43).

**Morphology:****Grossly:**

Malignant Brenner tumors are 16–20 cm in greatest dimension on average. They are cystic and solid with papillary or polypoid components. A gritty texture on cut surface may represent prominent calcifications (43).

**Microscopically:**

Malignant Brenner tumors have an exuberantly proliferative transitional epithelium with cellular atypia and stromal invasion. The malignant component consists of large, closely packed irregular nests of transitional epithelial cells infiltrating into the surrounding stroma. A desmoplastic stromal reaction will help identify the areas of invasion. The epithelial cells demonstrate nuclear pleomorphism with hyperchromasia and numerous mitoses. Foci of necrosis are commonly seen. Calcifications are often prominent and squamous differentiation has been reported (44).

**Immunohistochemical profile**

Ovarian transitional cell carcinoma is negative for CK20, thrombomodulin, and uroplakin, while vimentin, CA-125, WT1 are positive in primary ovarian transitional cell carcinoma (45).

**Molecular pathology:**

All Brenner tumors are negative for TP53 mutations. These tumors display the presence of sporadic gene mutations, mostly occurring in genes involved in cell cycle control, DNA repair and epigenetic control. EGFR-RAS-MAPK mutations, Exon 9 PIK3CA mutations, P16 LOH, and Ras driver mutations have been described in malignant Brenner tumors. The analysis of copy number alterations showed that 75% of the malignant Brenner tumors displayed Mouse double minute 2 homolog (MDM2) modifications, in one case associated with Cyclin D1 (CCND1) amplification and this support that malignant Brenner tumors are genetically different from ovarian cancers with transitional-like cell morphology (46).

**Transitional-like high-grade serous carcinoma (TLHGSC)**

It is a variant of high-grade serous carcinoma of the ovary. It was previously classified under the same umbrella as Brenner tumors (transitional cell tumors of the ovary) and was called transitional cell carcinoma. They frequently have a papillary architecture with a multilayered transitional cell epithelium and smooth luminal borders. A nested pattern similar to that seen in malignant Brenner tumors can be seen. TLHGSC is usually strongly positive for p16, PAX8, and WT1 and shows p53 staining consistent with a p53 mutation, and is negative for EGFR, cyclin D1, and Ras (43).

**Malignant mixed Mullerian tumors carcinosarcoma****Incidence:**

Malignant-mixed mullerian tumors carcinosarcomas are rare tumors that account for only 1%–2% of all malignant ovarian tumors (47).

**Morphology:****Grossly:**

These tumors are bilateral in one third of cases. They are usually large (15-20 cm mean diameter), friable, solid and/or cystic with areas of hemorrhage and necrosis. Occasionally bone or cartilage can be palpated (44).

**Microscopically**

Malignant mixed Mullerian tumors are mixed tumors composed of sarcomatous and carcinomatous components. The sarcoma-like elements may have the appearance of chondrosarcoma (the most common), osteosarcoma, rhabdomyosarcoma, or angiosarcoma, the carcinomatous component often consists of adenocarcinoma, and squamous cell carcinoma is extremely rare (47).

### **Sex determining region Y-box 2 (SRY)-box 2 (SOX2)**

Sex determining region Y-box 2 (SRY)-box 2 (SOX2) is one of the main members of the Sox family which consists of at least 20 members that are divided into 8 groups (from A to H, based on their high mobility group (HMG) sequence identity in humans). SOX2 is considered as one of the key founding members of core pluripotency associated transcription factors and has a great role in self renewal and maintenance of stemness of embryonic- and neuronal stem cells (SCs), reprogramming somatic cells into induced pluripotent stem cells (iPSCs), and in regenerative medicine. Recently, SOX2 amplification, usually couples with increased expression, were found in various human cancers, including breast, lung, esophagus, colon, prostate and ovarian cancer as well (2).

### **SOX2 structural organization**

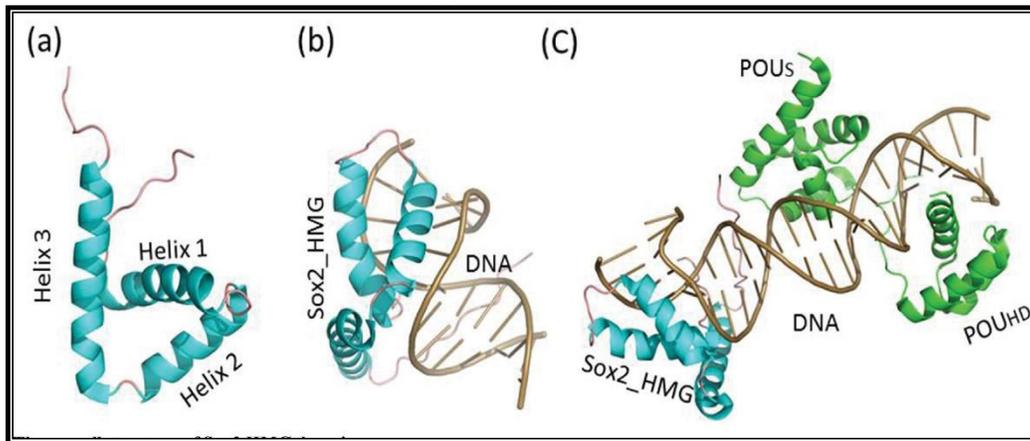
The human Sox2 protein is composed of 317 amino acids (~35 kD). 20 different amino acids, including alanine (8.2%), glycine (11.0%), leucine (6.3%), serine (11.4%) and Methionine (7.9%). The protein contains three primary domains; a short N-terminal domain, a DNA binding HMG domain, and a C-terminal trans-activation domain. The HMG domain comprises of roughly 70 amino acids, forming a three-helix sandwich between N- and C-terminal domains (48).

The concave surface forms the docking area for binding the DNA with a sequence-specific recognition. The binding to the specific target DNA site is enabled via the minor groove (especially helix 1 and helix 2 forming extensive contacts with the DNA in this groove) and inducing a large conformational change in DNA. The conformational change also helps in the unwinding of DNA, which helps in recruiting other factors. This unwinding also makes the minor groove shallow which is compensated by the compression of the major groove of DNA. The Sox2-HMG domain itself undergoes a minor change after binding to DNA. The specificity of these interactions is mainly mediated by numerous base pair specific hydrogen bonds (49).

The C terminus of the Sox2- HMG domain remains unstructured in the absence of an interacting protein partner, playing an important role for ternary complex formation (HMG/POU/DNA), because the interaction of this portion to the minor groove increases the HMG/DNA surface. Although Sox2 is able to bind to DNA on its own, it is converted to a high-affinity ligand binder in the presence of other DNA-binding protein partners, as part of a ternary complex (50).

The C-terminal portion of Sox2 is mainly unstructured resulting in the lack of availability of a full-length structure. This portion is shown to have key functional roles as evident in various studies. The last 71 C-terminal amino acids of Sox2 are required for the interaction with  $\beta$ -catenin. This interaction is important for  $\beta$ -catenin activation and stabilization which induces Wnt signaling pathway inhibition (51).

The C-terminal of Sox2 along with N-terminal also participates in its association with another transcription factor Sall4 (sal-like protein 4), to form nuclear protein complexes. Sox2 interacts directly with members of the groucho family of corepressors like Grg (groucho related gene) via its C-terminal domain. The role of the C-terminal region and the HMG domain is clear in mediating protein-protein interactions (52).



**Figure (1):** The overall structure of Sox2 HMG domain. (a) Representation of Sox2 HMG domain containing three helices (b) Ribbon diagram of Sox2 HMG bound to the recognition DNA sequence (c) The cooperation between the Sox2 HMG domain and POU domain facilitates each other binding on DNA surface. The POU domain has two subdomains POU5 (specific) and POUHD (homeodomain) (48).

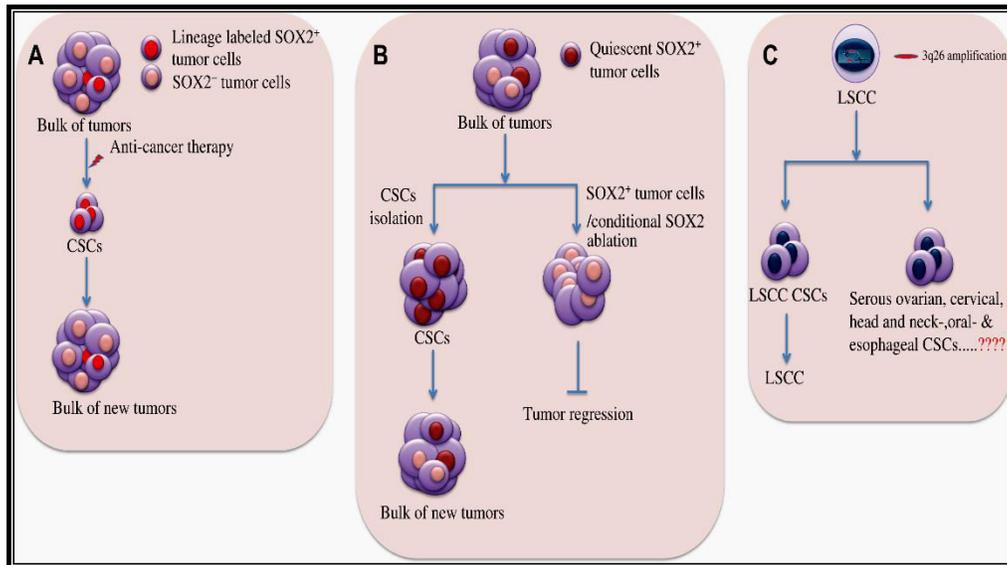
SOX2 gene is located on chromosome 3q26.33, encoding a 34-kDa transcription factor. Its expression is mainly found in stem cells and many different kinds of cancer cells, including glioma, breast cancer, colorectal cancer and etc. SOX2 protein expression locates mostly nuclear (73.9%) and less extend cytoplasmic (4.3%). So SOX2 is considered a nuclear protein (2).

SOX2 functions as an activator or suppressor of gene transcription as this protein family shares the highly conserved DNA binding domains known as HMG (High-mobility group) box domains which permits highly specific DNA binding (53).

### SOX2 and cancer stem cells (CSCs)

Sox2 is generally known as a stem cell determining transcription factor, and it is required for the maintenance of stem cells. High level of SOX2 expression is a key in conferring stem cell like phenotypes to more than a dozen of tumors. Cancer stem cells (CSCs) are found in various cancer types which initiate tumor formation and metastasis. There are

always several distinct CSC subpopulations which are resistant to chemoand radiotherapy (54).



**Figure (2): SOX2 and cancer stem cells :**(A) Anti-tumor therapy kills most of the cancer cells, leaving behind SOX2<sup>+</sup> tumor cells that serve as CSCs for tumor regrowth. Lineage tracing experiments showed that developmental hierarchies were preserved, and SOX2<sup>+</sup> tumor cells were therapy resistant and responsible for tumor progression. (B) Enrichment of quiescent SOX2<sup>+</sup> tumor cells from tumor masses, which seed for new tumors containing both SOX2<sup>+</sup> and SOX2<sup>-</sup> tumor cells (left). Lineage-specific ablation of SOX2<sup>+</sup> tumor cells or conditional SOX2 deletion leads to tumor regression (right). (C) High SOX2 and PRKCI expressions in LSCC gain via 3q26 chromosomal amplification. Coordinated overexpression of both of these proteins is attributed to LSCC stemness and enhanced tumorigenicity. Since 3q26 copy number gains are the most frequently occurring mutation in SOC, cervical, head and neck, oral, and esophageal carcinomas, it might be involved in the generation of respective CSCs (49).

These characteristics due to Sox2 deletion can be reversed by an immunotherapeutic approach based on Sox2 peptides, demonstrating a Sox2-directed strategy to eradicate tumor-initiating cells. As Sox2 is required for the maintenance of neural stem cells, they are also required for the neural cancer stem-like cells. The aberrant proliferation and differentiation have been observed in Sox2-deleted tumor cells, which are also highly sensitive to external stimuli leading to an increase in cell death (48).

A similar role of Sox2 has been reported in cases of lung adenocarcinoma where the functions of Sox2 were analyzed in cancer-initiating cells (CICs) derived from human lung adenocarcinoma. Sox2 is also associated with tumor initiation and propagation in epithelial ovarian cancer (EOC) (52).

**The signaling pathways of SOX2 in cancer progression and metastasis**

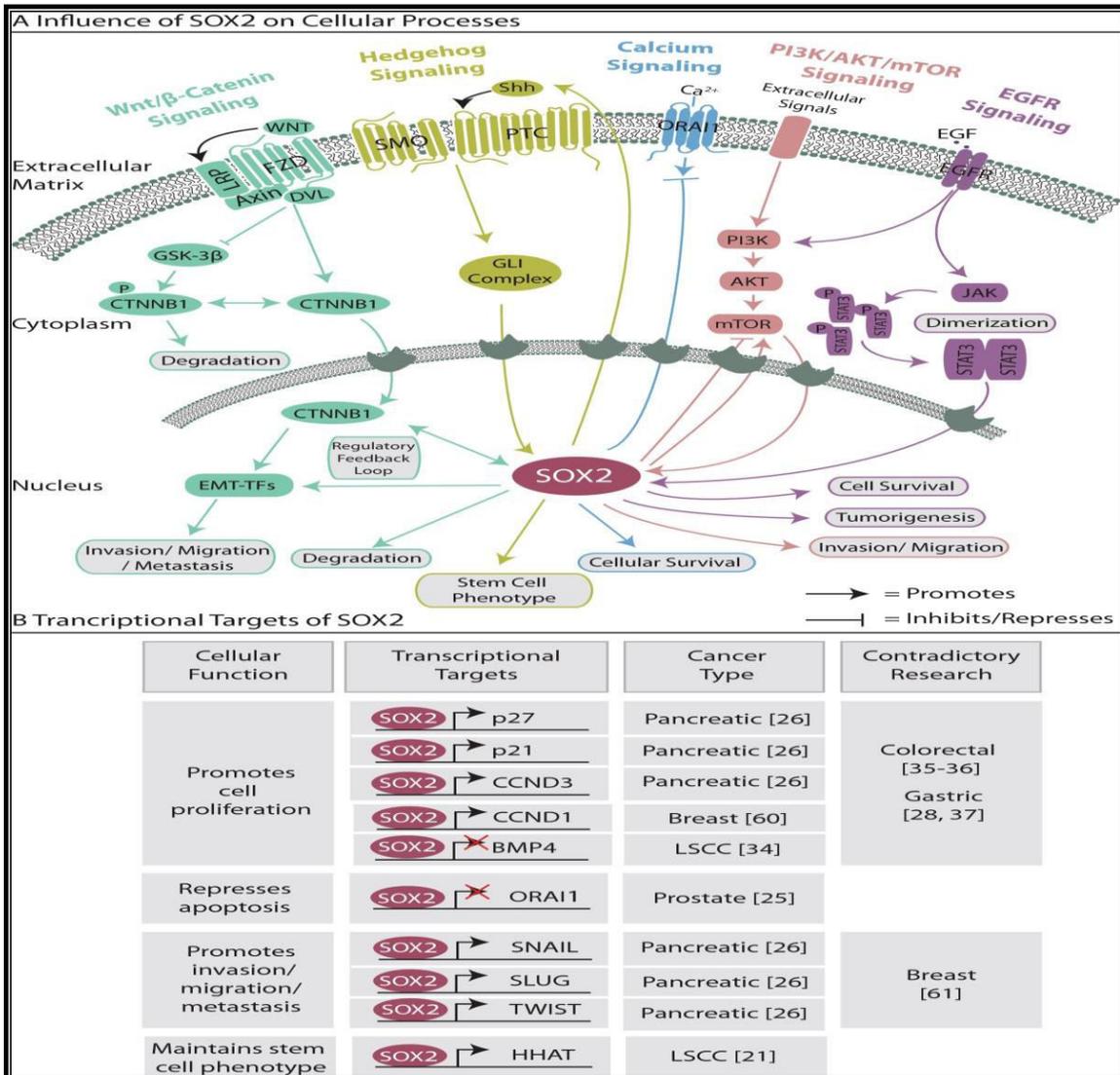
SOX2 expression has been reported at both the RNA and protein levels for many tumors, data available from The Cancer Genome Atlas indicates that SOX2 mRNA is elevated in many cancers, relative to normal tissue. Dysregulation of SOX2 expression is an important factor contributing to cancer pathogenesis. Amplification of the SOX2 gene locus and an increased SOX2 expression in turn affects cancer progression (48).

SOX2 controls several features of cancer cells such as proliferation, migration, invasion, metastasis and tumor initiation and this progression is associated with epithelial mesenchymal transition (EMT), which is characterized by the loss of the cell to cell contact of epithelial cells and degradation of the surrounding matrix to enable invasion and metastasis. EMT helps in generating the heterogenous subpopulation, endowing it towards tumor propagation and can be distinguished by a reduction in epithelial markers or acquisition of mesenchymal markers (55).

Various regulatory factors and signaling pathways are known to play a central role in tumor propagation like Wnt/ $\beta$ catenin signaling pathway. The role of Sox2 in improving the metastasis potential of breast and prostate cancers was shown by promoting EMT through Wnt/ $\beta$ -catenin activation. Recently, similar results were obtained in case of tongue squamous cell carcinoma (TSCC), with Sox2 overexpression was reported to be associated with malignant phenotypes and EMT progression (52).

Many studies were able to show that the SOX2 locus is amplified in esophageal and lung squamous cell carcinomas (SCCs), which results in increased SOX2 expression enhancing proliferation and anchorage-independent growth of SCCs. In non-small cell lung cancer, the ability of SOX2 for self-renewal depends on EGFR-Src-Akt signaling and shows EMT characteristics.

This signaling pathway regulates Sox2 expression via TGF- $\alpha$  and leads to cell proliferation and evasion of apoptosis. In pancreatic ductal adenocarcinoma, SOX2 is rarely expressed cases of in pre-malignant pancreatic intraepithelial neoplasia, but its expression has been reported to increase to 60% in cases of poorly differentiated and neurally invasive components (56).



**Figure (3): Influence of SOX2 on oncogenic-related processes and transcription. (A)** SOX2 is an important regulator of cellular processes related to cancer. Some of these processes include but aren't limited to WNT/ $\beta$ -CATENIN signaling, EMT and JAK/STAT3 signaling. In most cases, SOX2 functions downstream in the nucleus. SOX2's activity leads to further downstream effects and finally alters cellular phenotypes such as cellular survival, invasion and metastasis. **(B)** SOX2 is typically regulating processes downstream on a transcriptional level. There are several examples of SOX2 influencing cancer phenotypes by repressing or activating particular target genes including EMT promotion via binding to the promoter regions of SNAIL, SLUG and TWIST. Therefore, SOX2 functions in cancer as a key transcription factor (53).

### **Role of SOX2 expression in prognosis and survival**

The level of SOX2 expression in a tumor represents a prognostic factor to determine the clinical outcome for a cancer patient as some studies indicate that patients with tumors with SOX2 amplification relapse significantly more often and there was a tendency for a lower overall survival rate for SOX2-amplified patients. Many studies correlate high SOX2 expression in different cancer types with a poor prognosis, among them breast, oral/tongue, esophageal, lung, hepatocellular, colorectal, prostate cancer, nasopharyngeal and sinonasal carcinoma (57).

In addition to survival and recurrence, high SOX2 expression has been linked to the infiltrative and metastatic capacity of tumor cells. In cases of colorectal cancer and prostate cancer, SOX2-expressing tumors have been shown to correlate with increased distant and lymphatic metastases.

And also in esophageal squamous cell carcinomas, tumors in which more than 50% of the cells express SOX2 were correlated with increased lymphatic and vascular invasion, poor differentiation, and incomplete surgical resection (56).

### **SOX2 and drug resistance**

Malignant cells can become resistant to many anti-cancer drugs by several ways which makes finding a solution to this problem more difficult (58).

They may enter a 'drug-tolerant state' that could help them to survive and develop resistance against the drug. The penetrance of CSCs in addition to their long-term self-renewal ability is related to the resistance to the conventional anti-cancer therapies and these surviving therapy resistant CSCs have potential to serve as the precursors of newly formed tumor masses, eventually leading to clinical relapse (49).

SOX2 is involved in major mechanisms of therapeutic resistances in cancers, which lead to clinical relapse as it catalyzes pro survival and anti-apoptotic signaling in diverse range of cancers.

For example, SOX2 expression develops resistance to commonly used drugs in lung cancer (e.g., cisplatin, paclitaxel) by suppressing pro-apoptotic BH3-only genes, namely BIM and BMF and through activation of oncogenic EGFR and BCL2L1 signaling. CSC subpopulation exhibit the aspects of EMT-program activation with an increase in tumor initiating and migrating capacities and the activation of EMT-program mediates drug resistance (2).

Focusing on the role of SOX2 in drug resistance and development of drugs targeting SOX2 could greatly improve the treatment options for patients with a multitude of cancers and provide better therapeutic regimens especially those with highly refractory tumors (59).

**SOX2 in Ovarian cancer:**

SOX2 is considered to be an important prognostic factor for high grade ovarian cancer patients, where SOX2 expression has an impact on both progression-free, and overall survival (60)

SOX2 functions as an oncogene in epithelial ovarian cancer cells by promoting CSC-like characteristics including spheroid formation, cell proliferation, cell migration, drug resistance, and tumorigenic potential (61).

ovarian cancer, several studies have proved that the expression of SOX2 gradually increases from benign and borderline to malignant ovarian tumors. Positive correlations between SOX2 expression and disease stage and tumor differentiation have been reported (62) and SOX2 expression has also been associated with the poor clinical outcome of ovarian cancer (61)

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