

Original Article

Role of SOX9 protein in ovarian carcinoma: A molecular insight

Ramoju Harshitha,¹ Lajya Devi Goyal,² Monica Kakkar,¹ Himanshu Sharma¹ & Gitanjali Goyal¹

Departments of ¹Biochemistry, and ²Obstetrics and Gynecology, All India Institute of Medical Sciences Bathinda, Punjab, India

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Background and objectives: Ovarian carcinoma is one of the most lethal carcinomas among females. Its high prevalence and shorter 5-year survival rate is due to the fact that most of the cases are diagnosed at later stages. This highlights the importance of early diagnosis through reliable biomarkers. We studied the diagnostic role of SOX9 protein in ovarian carcinoma and its diagnostic ability. The primary objective was to compare the level and clinical relevance of SOX9 protein in the tissues of patients with ovarian carcinoma with non-malignant ovarian tissues.

Methods: Tissue levels of SOX9 protein were estimated in the study and control groups (60 each group). SOX9 levels were compared between the study vs. control groups and also between high grade and low-grade ovarian cancer. SK-OV3 ovarian adenocarcinoma cell line was used as supportive evidence to prove the presence of SOX9 in malignant ovarian cells.

Results: Levels of SOX 9 protein (3.9 ± 2.7 ng/mL) were high in tissue of ovarian cancer patients when compared to non-malignant (1.5 ± 1.1 ng/mL) ovarian tissues. Higher levels of SOX 9 protein were found in tissues of ovarian cancer patients when compared to non-malignant ovarian tissues. The mean of SOX 9 levels in tissues of high-grade serous carcinoma was 3.5 ± 2.5 ng/mL as compared to 1.0 ± 0.9 ng/mL in low-grade serous carcinoma.

Interpretation and conclusions: SOX9 appears to be an important player in the molecular tumourigenesis of ovarian cancer, particularly in high grade tumours.

Keywords Malignant; Ovarian cancer; SOX9; Tissue lysate; Tumour marker

Ovarian carcinoma is sixth most common cancer affecting women in various parts of the world, causing highest number of deaths every year than any other female genital cancers.¹ Along with the detailed history and gynaecological examination, the current diagnostic technologies commonly used are tumour markers like CA 125, HE4, CA 19-9, LDH, AFP, osteopontin, mesothelin, kallikreins, and modalities like transvaginal/abdominal ultrasound, CECT, PET-CT, MRI, followed by biopsy and histopathological confirmation. There are various algorithms to calculate risk of malignancy like RMI (Risk of Malignancy Index), ROMA (Risk of Ovarian Malignancy Algorithm), and IOTA (International Ovarian Tumor Analysis).² Among serum biomarkers, CA125 or HE4, have better specificity and sensitivity when used in

combination. There is a need for novel and reliable biomarkers to improve early diagnostic technology.

SOX group of transcription factors were discovered in mammals in 1990. SOX9 is a transcription factor protein which is encoded by *Sox9* gene. It has a role in sexual development, cellular differentiation, regulating chemo resistance in tumour cells, functioning as a tumour suppressor in some cancers and implicated to be an oncogene in few cancers.³⁻⁵

The objective of the present study was to investigate the expression of SOX9 protein in ovarian carcinoma tissues compared to non-malignant ovarian tissues and to explore its association with tumour grade. We also aimed to provide *in vitro* evidence using SK-OV3 ovarian carcinoma cell line, thereby establishing the

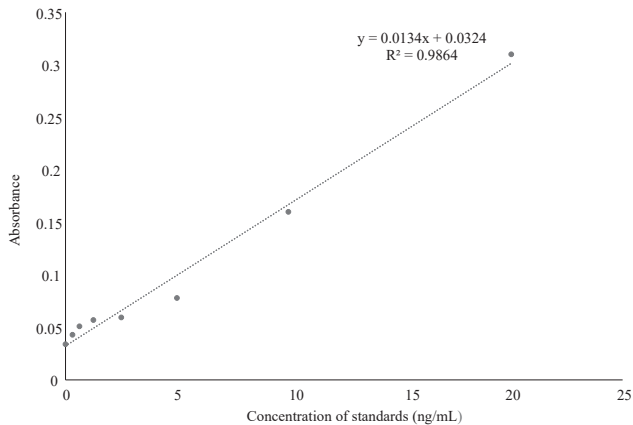


Fig. 1. Standard curve of ELISA of SOX 9 standards of different concentrations. X-axis shows concentration of standard and y-axis shows absorbance. The equation and R^2 value displayed on the graph. Using the equation, concentration of SOX 9 protein was estimated in all the samples including the tissue lysates and cell lysate.

biological relevance of SOX9 as a potential marker for ovarian cancer.

Methods

A cross-sectional study was conducted by the department of Biochemistry, All India Institute of Medical Sciences Bathinda, Punjab, India on patients diagnosed with ovarian carcinoma by histopathological evidence during the study period (October 2022 to June 2024), after ethical clearance from the Institutional Ethics Committee.

Participants and samples:

Study participants: 60 study group participants and 60 age matched controls were enrolled based on hospital statistics and the number of ovarian surgeries during the study period. Study group included females of all age groups with histopathological evidence of ovarian carcinoma and posted for surgery; excluding pregnant and lactating women, patients having other chronic illness or other malignancies affecting SOX9 (hepatocellular carcinoma, breast carcinoma, pancreatic cancer), and advanced cases where surgical resection is not possible. After recruiting the study participants, fresh ovarian tissue sample was collected after the surgery for all the cases and controls, along with that blood sample is collected under aseptic measures for CA125 estimation.

Ovarian tissue was washed with phosphate-buffered-saline (PBS) to clear any blood residues and then weighed. The tissue was then minced into uniform

mixture in PBS. For 1 g of tissue, 9 mL of PBS was added for homogenization. Further homogenisation was done using tissue homogeniser. The whole process was done on ice for maximal yield.

Cell lines and reagents: To prove the presence of SOX 9 in ovarian cancer cells, SK OV3 cell lines were employed for the experimental workup on SOX 9 protein. SK-OV 3 cell-lines were obtained from NCCS (National Centre for Cell Science), Pune and cultured according to manufacturer's instructions. SK-OV3 cells were cultured in the medium of McCoy's 5A which was supplemented with 10% of foetal bovine serum. Cells were cultured in a humidified environment comprising 5% CO₂ and 95% air. Cells were 90% confluent in 6 days after each passage. Cells from 3 consecutive passages were used for the experiment after lysis. Cells were washed with ice cold PBS before they were lysed using lysis buffer.

Estimation of total protein in the lysates by BCA method: BCA (bicinchoninic acid) method is highly sensitive and detergent-compatible approach for the colorimetric detection and measurement of total protein by the reduction of Cu⁺⁺ to Cu⁺ by protein in an alkaline medium. This estimation was done using BCA protein estimation kit from GeNei Laboratories Pvt Ltd. A standard curve was generated and the R^2 value was found to be 0.99.

Estimation of SOX 9 protein by ELISA: SOX9 protein in tissue extract obtained from study samples and the cell lysates from SK OV3 cells was determined by ELISA method using human transcription factor SOX-9 ELISA Kit (BioTechno Labs). A standard curve of SOX9 was plotted as shown in **Figure 1**.

Estimation of CA125: Serum levels of CA125 were measured by chemiluminescence microparticle immunoassay.

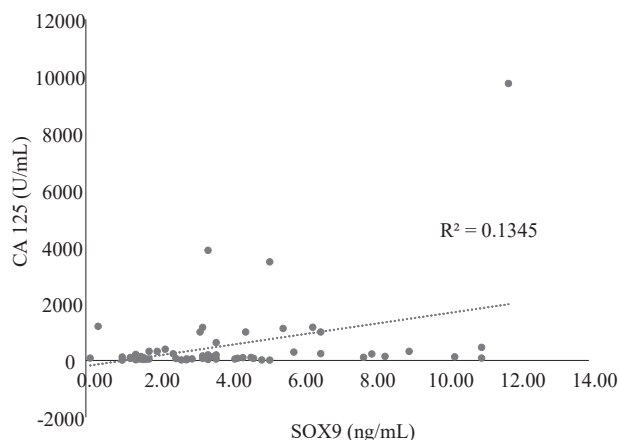
Statistical analysis: The descriptive statistics are reported as mean \pm SD for normally distributed variables and as median (IQR) for skewed data. Normality was assessed using the Kolmogorov–Smirnov test. For comparisons between two independent groups (e.g., SOX9 levels in cases vs. controls; high-grade vs. low-grade carcinoma), an unpaired Student's t-test was used for normally distributed variables. Associations between categorical variables (e.g., menopausal status and malignancy risk) were evaluated using the Chi-square test. Correlations between continuous variables (e.g., SOX9 and CA125) were analysed using Pearson's correlation coefficient.

Table I. Comparison of age and tissue protein levels in ovarian carcinoma cases (n=60) and non-malignant controls (n=60); and deviation, minimum, maximum, and P-values indicating statistical significance of case-control comparisons

Variable	Cases (n=60) Mean (SD)	Controls (n=60) Mean (SD)	P value
Age (yr)	45.4 (14.0)	44.2 (13.2)	0.63
Tissue protein by BCA ($\mu\text{g}/\text{mL}$)	718.5 (209.5)	429.7 (192.4)	<0.0001
SOX9 protein (ng/mL)	3.9 (2.7)	1.5 (1.1)	<0.0001

Table II. Comparison of biomarker levels between high-grade and low-grade serous ovarian carcinoma patients

Biomarker levels	High grade serous carcinoma, n=38	low grade serous carcinoma, n=6	P value
CA 125 (U/mL)	613.9 (138.7)	36.0 (19.1)	<0.0001
BCA ($\mu\text{g}/\text{mL}$)	670.5 (220.5)	714.8 (262.5)	0.66
SOX 9 (ng/mL)	3.5 (2.5)	1.0 (0.9)	0.019

**Fig. 2.** Showing the scatter plot of CA125 and SOX 9 among the patients of ovarian cancer, showing a positive correlation between them.

Results

We have enrolled 60 cases with ovarian carcinoma and 60 controls based on the eligibility criteria. Of 60 cases with ovarian carcinoma, majority (n=44,73%) had serous carcinoma. Mucinous carcinoma germ cell tumour and others were diagnosed in 5, 6 and 5 patients, respectively. Of 60 patients 37 (61.7%) were post menopausal. Menopausal status was significantly associated with risk of malignancy (OR 2.41; 95% CI: 1.15-5.02; $P=0.018$).

Tissue protein levels in study group vs. control group are presented in **Table I**.

Median (IQR) of CA125 levels in study group was 116.5 (54.9-319.25 U/mL). The mean level of CA125 levels in tissues of high-grade serous carcinoma was 613.9 ± 138.7 U/mL compared to 36.0 ± 19.1 U/mL

in low grade serous carcinoma ($P<0.001$). **Table II** compares various biomarker levels between high-grade and low-grade serous ovarian carcinoma.

Correlation of SOX 9 protein levels with CA 125: CA125 and SOX 9 levels in the study group were found to have positive correlation coefficient of 0.37 and P value of 0.004 as in **Figure 2**. CA 125 was significantly associated with SOX 9 levels. There is a significant positive expression of SOX 9 protein in the ovarian cancer cell line, acting as supportive evidence for the findings of increased SOX 9 protein in ovarian cancer tissues.

Cell lysate as supportive evidence to prove the presence of SOX9 in malignant ovarian cells: In SK-OV3 ovarian carcinoma cell lysates, the total protein concentration measured by BCA assay was $838.5 \mu\text{g}/\text{mL}$, $675.3 \mu\text{g}/\text{mL}$ and $638.5 \mu\text{g}/\text{mL}$ in the consecutive passages. Corresponding SOX9 protein levels measured by ELISA were 6.5 ng/mL, 5.3 ng/mL and 4.0 ng/mL, respectively. These findings provide supportive *in vitro* evidence of SOX9 expression in malignant ovarian cells.

Discussion

SOX9 is a nuclear protein which is regulated by the gene *Sox 9* that controls cell differentiation and pluripotency and regulates tissue homeostasis in adults.⁶ The present study concluded that SOX 9 protein is significantly overexpressed in ovarian carcinoma tissues when compared to the non-malignant controls, with markedly higher levels in high-grade tumours. SOX9 expression also showed a positive correlation with CA 125 levels and was consistently expressed in SK-OV3

ovarian carcinoma cells. Collectively, these findings highlight the biological relevance of SOX9 in ovarian tumourigenesis and its potential as a diagnostic and prognostic biomarker.

Several studies support our findings that SOX9 is upregulated in malignant ovarian tissues. Jo A *et al*⁷ and Sherman-Samis *et al*⁸ have reported higher expression of SOX 9 levels in high grade ovarian carcinoma, where it was associated with poor clinical outcome, also suggested the use of SOX 9 as a prognostic tool and perhaps a novel therapeutic target for further exploration. Similarly, Malki *et al*⁹ demonstrated SOX9 induction in ovarian cancer cell lines and confirmed its presence in human tumours.⁹ Siu *et al*¹⁰ showed that signalling cascades involving Erk1/2 and Nanog can drive SOX9 overexpression, contributing to invasion and metastasis. Recent evidence indicates that SOX9 is involved in chemoresistance through miR-34c/SOX9 regulatory axis, further indicating its clinical relevance.^{11,12}

SOX9 is known to influence multiple oncogenic pathways. Accumulating evidence shows their involvement in tumour initiation and progression. Onder *et al*⁶ evaluated immunohistochemistry markers such as FOXL2, SOX9 and β -catenin status in differentiating sex cord tumour with annular tubules (SCTAT) from other sex-cord stromal tumour and concluded that diffuse and strong expression of SOX 9 was a distinguishing feature.⁶ This strongly implicates the diagnostic utility of SOX9 in these tumours.¹² Raspaglio *et al*¹³ observed that higher levels of SOX9 are seen in biopsies of human Sertoli tumours, where its co-expression with Ki-67 and BCL-2 suggested a link with proliferative and anti-apoptotic signalling. Substantially, they reported that overall survival is significantly affected by SOX9 expression.¹³

Mechanistically, SOX9 modulates PGD2 linked pathways, represses Wnt/ β -catenin signalling and cooperates with Snail/Slug to induce EMT (epithelial mesenchymal transition), thereby enhancing invasiveness.¹⁴⁻¹⁶ Our findings of elevated SOX9 in high grade tumours align with these mechanisms, suggesting that its overexpression may accelerate the tumour progression and resistance to apoptosis. Aldaz *et al*¹⁷ further implied that the oncogenicity of SOX9 is through the upregulation of BMI 1 expression which represses the tumour suppressors like p21CIP and p16INK4A. SOX 9 induces tumour progression by promoting cell proliferation and evading senescence and apoptosis. Silencing of SOX9

showed reduced cell viability and cell proliferation and induced senescence, abrogating proliferation of ovarian cancer cells.¹⁸

It is also important to acknowledge that SOX9 exhibits context-dependent roles, functioning as an oncogene in ovarian and breast cancer, but reported as a tumour suppressor in cervical cancer.^{19,20} This duality underscores the complexity of SOX9 biology and the need for tissue-specific investigations. In ovarian carcinoma however, the preponderance of evidence, including our results, support its oncogenic activity. SOX 9 has the potential to be the novel marker, but the translation into a diagnostic or prognostic tool would further require validation, including studies on serum or minimally invasive tissue specimen, larger sample size and longitudinal study designs.

The strengths of our study include the use of both clinical tissue samples and SK-OV3 cell lines, ensuring translational relevance and reproducibility of SOX9 expression. The present study also integrated correlation with CA 125, a standard biomarker which strengthens the findings. Limitations include relatively small sample size, the cross-sectional design, and the restriction to tissue-based measurements, which precludes direct application as a non-invasive diagnostic tool. Further studies should evaluate SOX9 in serum, ascitic fluid or circulating tumour cells to validate its clinical utility in early detection and monitoring.

In summary, our findings reinforce SOX9 as an important player in the molecular tumourigenesis of ovarian cancer, particularly in high grade tumours, where its expression correlates with disease severity. The high levels of SOX9 in early stages can prove its diagnostic ability with a sensitivity of 81%. By linking SOX9 with established oncogenic pathways such as Wnt/ β -catenin inhibition, EMT promotion and BMI1 mediated repression of tumour suppressors, our study provides a mechanistic context to its overexpression in ovarian carcinoma. SOX 9 is yet to be explored as a therapeutic target, meriting its further investigation in larger, multicentric cohort studies.

Author contributions: RH: Sample collection, conducted the experiments, results analysis, manuscript writing; LDG: Provided clinical samples; MK: Critically revised the manuscript; HS: Conducting cell culture experiments; GG: Conceptualized and designed the experimental study, supervised, statistical analysis, and manuscript writing. All authors have read and approve the final printed version of the manuscript.

शोध-संदेश

यह अध्ययन महिलाओं में पाए जाने वाले जानलेवा अंडाशय कैंसर (ओवेरियन कार्सिनोमा) में SOX9 प्रोटीन की भूमिका और इसके निदानात्मक महत्व पर केंद्रित है। चूंकि अधिकांश मामलों का पता देर से चलता है, इसलिए प्रारंभिक अवस्था में विश्वसनीय बायोमार्कर की आवश्यकता होती है जिससे रोग की पहचान हो सके। इस शोध में ओवेरियन कैंसर रोगियों के ऊतकों में SOX9 के स्तर की तुलना गैर-कैंसरग्रस्त अंडाशय ऊतकों से की गई। परिणामों से स्पष्ट हुआ कि विशेषकर उच्च-ग्रेड ट्यूमर में SOX9 की अभिव्यक्ति अधिक होती है और यह रोग की गंभीरता से संबंधित है। प्रारंभिक चरणों में SOX9 का उच्च स्तर 81% sensitivity के साथ इसके निदानात्मक उपयोग को दर्शाता है। अतः SOX9 को संभावित निदानात्मक एवं भविष्य में चिकित्सीय लक्ष्य के रूप में मानते हुए बड़े, बहु-केंद्रित अध्ययनों, और अधिक शोध की आवश्यकता है।

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Conflicts of Interest: None.

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For correspondence: Dr Gitanjali Goyal, Department of Biochemistry, All India Institute of Medical Sciences, Bathinda 151 001, Punjab, India
e-mail: gitanjaligoyal@yahoo.co.in