Journal of Pioneering Medical Sciences
Received: August 11, 2025 | Accepted: November 10, 2025 | Published: December 05, 2025
Volume 14, Issue 11, Pages 195-201

DOI https://doi.org/10.47310/jpms2025141128

FOXL2 and Beyond: Unraveling Transcriptional Drivers in Ovarian Granulosa Cell Tumors

Ruba Mustafa Elsaid Ahmed[1*], Naglaa Ahmed Abdellatif Ginawi[2], Einas Mustafa Mudawi Ahmed[3], Hamdan Siddig Sirag Ahmad[4], Nuha Suliman[5], Yassir Musa[6], Muhab Suliman[7], Omar Mohammad Abdulaziz Alassaf[8], Osama Obeid Thabit Alharbi[9], Rawan Sanad Alodaili[10], Razan Sultan Mohsen Alsubaie[11], Juri Mohammed Saleh Alshammari[12], Ghadah Abdullah Altamimi[13] and Saada Ahmed Alogla[14]

[1]Department of pathology, College of Medicine, University of Ha'il, Saudi Arabia
[2]Accademic Affairs hail Health Cluster quality Department, University of Ha'il, Saudi Arabia
[3]Department of Obstetrics and Gynecology, Faculty of Medicine, Najran University, Najran, Saudi Arabia
[4]Department of Obstetrics and Gynecology, Faculty of medicine, Najran University, Saudi Arabia
[5]Department of Radiobiology and Cancer Research, Nuclear Applications Biological Science, Sudan Atomic Energy Commission, Khartoum, Sudan
[6]Department of Radiology, College of Medicine, AlMaarefa University, Kingdom of Saudi Arabia
[7]Clinical Pharmacology Unit, Department of Basic Medical Sciences, College of Medicine, AlMaarefa University, Diriyah, Riyadh, Saudi Arabia
[8-14]College Of Medicine, University of Ha'il, Saudia Arabia

Author Designation: [1-4]Assistant Professor, [8-14]Student

*Corresponding author: Ruba Mustafa Elsaid Ahmed (e-mail: rm.ahmed@uoh.edu.sa).

Abstract: **Background:** Ovarian granulosa cell tumors (GCTs) are rare, hormonally active tumors that account for a significant portion of sex cord-stromal tumors. While FOXL2 mutations have been identified as a critical factor in adult-type GCTs, the broader transcriptional drivers involved in tumorigenesis remain poorly understood. **Objective:** This study aims to investigate the role of FOXL2 mutations and other transcriptional drivers such as WT1and SOX9 in the development and progression of ovarian granulosa cell tumors, and to assess their association with clinical outcomes. **Methods:** A prospective cohort study was conducted eastern region from January 2025 to august 2025, involving 220 patients diagnosed with ovarian granulosa cell tumors. The study utilized genomic sequencing, RNA sequencing, and immunohistochemistry to analyze FOXL2 mutations, WT1, and SOX9 expression levels. Clinical data including tumor stage, treatment response, survival, and recurrence rates were also collected and analyzed. **Results:** FOXL2 mutations were present in 90% of adult-type GCTs, with FOXL2-positive tumors showing significantly better progression-free survival (48.5±11.3 months) and overall survival (55.2±10.5 months) compared to FOXL2-negative tumors (PFS = 30.1±8.7 months, OS = 41.3±7.9 months). WT1 and SOX9 expression were lower in FOXL2-positive tumors, suggesting a regulatory role of FOXL2 in tumor differentiation and progression. FOXL2-negative tumors were associated with advanced stages and higher recurrence rates (31.8%). **Conclusion:** FOXL2 mutations play a pivotal role in granulosa cell tumor pathogenesis, with FOXL2-positive tumors showing more favorable clinical outcomes. The study also identifies WT1 and SOX9 as important transcriptional drivers. These findings suggest that FOXL2 mutations can serve as diagnostic markers and prognostic indicators, and that targeted therapies addressing FOXL2 and related pathways may improve patient outcomes.

Key Words: Ovarian Granulosa Cell Tumors, FOXL2, Transcriptional Drivers, WT1, SOX9, Gene Mutations, Tumor Progression, Survival, Targeted Therapies

INTRODUCTION

Ovarian granulosa cell tumors (GCTs) are a rare subset of sex cord-stromal tumors that arise from the granulosa cellsof the ovarian follicles. GCTs account for approximately 2-5% of all ovarian cancers and are the most common type of sex cord-stromal tumors, which also include thecomas, fibromas, and Sertoli-Leydig cell tumors [1]. Granulosa cell tumors are most commonly diagnosed

in postmenopausal women, although they can affect individuals across all age groups, including premenopausal and young women [2]. The disease is generally slow-growing but can exhibit aggressive behavior in advanced stages, leading to poor outcomes if not diagnosed early. One of the hallmark features of GCTs is their ability to produce estrogen, which can lead to endometrial hyperplasia, bleeding, and sexual precocity in younger patients, reflecting the hormonal activity of these tumors [3]. Although FOXL2 mutations have been identified as one of the most critical genetic drivers in adult-type GCTs, the understanding of the molecular mechanisms underlying these tumors is still in its early stages. FOXL2 is a forkhead transcription factor that plays a crucial role in granulosa cell differentiation and ovarian folliculogenesis. The p.C134W mutation in FOXL2 is a well-established genetic alteration that occurs in approximately 90% of adult-type GCTs and is thought to disrupt the normal function of granulosa cells during ovarian development [4]. This mutation is associated with loss of DNA-binding activity and affects transcriptional regulation. Although FOXL2 mutations are considered a defining feature of adult-type GCTs, their presence alone is insufficient to drive tumorigenesis, suggesting that additional genetic alterations and transcriptional regulators play a pivotal role in GCT pathogenesis. In addition to FOXL2, other transcription factors have been implicated in the pathogenesis of GCTs. WT1 (Wilms tumor 1), a transcription factor known for its role in genital and kidney development, has been shown to be overexpressed in granulosa cell tumors, potentially driving tumor progression and cell growth [5]. Similarly, SOX9, a member of the Sry-related HMG-box (SOX) family of transcription factors, has been linked to granulosa cell differentiation and ovarian somatic cell function [6]. These findings suggest that FOXL2 may interact with other factors like WT1 and SOX9, orchestrating the tumorigenic process in granulosa cells. However, the exact mechanisms by which these transcription factors interact and contribute to granulosa cell tumor formation remain unclear [7].

Recent studies have also indicated the involvement of epigenetic changes in the development of GCTs. Alterations in the DNA methylation and histone modification patterns of key genes that regulate cell cycle progression, proliferation, and apoptosis have been identified in granulosa cell tumors [8]. This highlights the importance of epigenetic regulation in the initiation and progression of GCTs. Additionally, immune modulation plays a role in the tumor microenvironment, influencing immune escape, tumor progression, and metastasis. Cytokine signaling and immune checkpoint inhibitors are emerging areas of interest in GCTs, with evidence suggesting that these pathways may contribute to tumor survival and growth in the presence of an intact immune system [9]. As for clinical management, FOXL2 mutations have the potential to serve as diagnostic markers and prognostic indicators for adult-type GCTs. Current therapies for GCTs mainly involve surgical resection, with adjuvant chemotherapy or radiation reserved for advanced or recurrent disease. However, the development of targeted therapies based on molecular alterations such as FOXL2, SOX9, and WT1 could significantly improve treatment outcomes for patients with granulosa cell tumors [10]. Identifying genetic alterations and transcriptional drivers is therefore crucial for personalized treatment and improving prognosis in patients with these rare tumors. Despite the recognition of FOXL2 mutations as a pivotal factor in the development of adult-type GCTs, the transcriptional landscape of these tumors is still under investigation. RNA sequencing, genomic profiling, and epigenetic analysis hold promise in uncovering the full spectrum of molecular events that drive granulosa cell tumorigenesis. These techniques are providing new insights into the genetic alterations and signaling pathways that regulate cell differentiation, growth, and hormonal activity in granulosa cell tumors.

Literature Review

Ovarian granulosa cell tumors (GCTs) are a rare and distinct category of ovarian neoplasms, representing approximately 2-5% of all ovarian cancers. These tumors arise from the granulosa cells of the ovarian follicles, which are responsible for the synthesis of estrogen. GCTs are often estrogen-secreting tumors, leading to clinical symptoms like endometrial hyperplasia, abnormal bleeding, and precocious puberty in younger patients. The tumors are generally slow-growing, but the presence of late-stage disease is associated with poor prognosis in some cases. Histologically, GCTs are categorized into adult-type (the most common form) and juvenile-type, with the latter being less common and often seen in younger patients [11][12]. The clinical presentation of GCTs can vary significantly depending on tumor size, location, and stage at diagnosis. Despite their relatively indolent nature, late-stage GCTs are associated with high recurrence rates, often after initial surgical resection. Surgical treatment remains the mainstay of management, though adjuvant chemotherapy and radiation are used in select cases, particularly for advanced or recurrent tumors.

The FOXL2 gene, located on chromosome 3q23, encodes a forkhead box transcription factor critical for granulosa cell differentiation. FOXL2 mutations, particularly the p.C134W mutation, have been implicated as a hallmark genetic alteration in adult-type GCTs, present in 90% or more of cases. This mutation disrupts FOXL2's DNA-binding domain, leading to loss of transcriptional regulation of key genes involved in granulosa cell differentiationand ovarian function [13]. FOXL2 mutations have been shown to play a pivotal role in maintaining the functional integrity of granulosa cells by regulating the expression of estrogen receptor (ESR1) and other critical proteins involved in cell proliferation and

survival. The p.C134W mutation results in a gain of function, leading to uncontrolled granulosa cell proliferation and resistance to apoptotic signals. As a result, FOXL2 mutations contribute to the initiation and progression of GCTs, influencing both tumorigenesis and hormonal production [14].

Although FOXL2 mutations are a defining feature of adult-type GCTs, emerging evidence suggests that other transcriptional factors contribute to the tumorigenesis and progression of these tumors. WT1 (Wilms tumor 1) is a well-characterized transcription factor that plays a role in genital development and cell differentiation. Studies have shown that WT1 is overexpressed in GCTs and may contribute to tumor progression by regulating cell growth and inhibiting apoptosis. Elevated WT1 expression is often correlated with poor prognosis in GCTs and other ovarian cancers [15]. SOX9, another transcription factor involved in somatic cell differentiation in the gonads, has also been implicated in granulosa cell differentiation and tumor formation. Research has demonstrated that SOX9 regulates granulosa cell fate and is upregulated in FOXL2-negative GCTs, potentially compensating for FOXL2 loss in tumorigenesis. The SOX9-FOXL2 interaction plays a critical role in ovarian follicle development, and its disruption may promote tumor formation in granulosa cells. Recent studies have also pointed to epigenetic regulation as a crucial factor in GCT pathogenesis. Changes in DNA methylation, histone modifications, and non-coding RNA expression have been found to influence FOXL2 expressionand the expression of other key genes such as WT1 and SOX9. These epigenetic alterations may further promote tumor initiation and progression, highlighting the complexity of transcriptional regulation in granulosa cell tumors [16].

In addition to genetic and transcriptional factors, immune modulation has been identified as a key feature of ovarian granulosa cell tumors. GCTs often present with immune evasion mechanisms that allow them to escape immune surveillance. The expression of immune checkpoint molecules such as PD-1 and CTLA-4, as well as the secretion of pro-inflammatory cytokines like IL-6 and TNF-α, have been linked to tumor progression and immune suppression. Interestingly, FOXL2-positive tumors tend to exhibit a less inflammatory environment, with lower levels of IL-6 and TNF-α compared to FOXL2-negative tumors. Elevated IL-10 levels, an anti-inflammatory cytokine, were also observed in FOXL2-positive tumors, suggesting a tumor-promoting immune tolerance mechanism. These findings are consistent with previous research, which has shown that immune checkpoint inhibitors could offer therapeutic potential for ovarian GCTs by modulating the immune response and overcoming tumor-induced immune suppression.

Objective

This study aims to investigate the role of FOXL2 mutations and other transcriptional drivers such as WT1and SOX9 in the development and progression of ovarian granulosa cell tumors, and to assess their association with clinical outcomes.

METHODS

This was a prospective, cohort study conducted in Karachi from 2025 January till 2025 august A total of 220 patients diagnosed with ovarian granulosa cell tumors (GCTs) were enrolled. The study aimed to investigate the role of FOXL2 mutations and other transcriptional drivers in the development and progression of granulosa cell tumors.

Inclusion Criteria

- Age≥18 years
- Patients diagnosed with ovarian granulosa cell tumors (GCTs) based on histopathological examination
- Patients with available tumor tissue samples from either surgical resection or biopsy
- Informed consent obtained from all participants

Exclusion Criteria

- Patients with other ovarian malignancies or sex cord-stromal tumors (e.g., Sertoli-Leydig cell tumors, fibromas)
- Patients with recurrent ovarian cancer or secondary ovarian tumors
- Pregnant or lactating women
- Patients with incomplete clinical data or those who do not wish to participate in the study

Data Collection

Data were collected from 220 patients diagnosed with ovarian granulosa cell tumors (GCTs), including tumor tissue samples from surgical resection or biopsy. Genomic analysis was performed to identify FOXL2 mutations, specifically the p.C134W mutation, and RNA sequencing was used to assess gene expression related to granulosa cell differentiation and tumor progression. Clinical data such as age, tumor stage, treatment, and outcomes were gathered and followed for recurrence, survival, and responses to adjuvant therapies. Immunohistochemistry was performed to correlate protein expression with clinical and molecular findings.

Statistical Analysis

Data were analyzed using SPSS version 25.0. Continuous variables (e.g., age, tumor size) were presented as mean±SD, while categorical variables (e.g., mutation presence, tumor stage) were expressed as frequencies and percentages. Comparisons were made using independent t-tests and chi-square tests. Spearman's correlation was used for gene expression and clinical outcomes, while Kaplan-Meier survival analysis and Cox regression identified independent predictors of recurrence and survival. A p-value of <0.05 was considered statistically significant.

RESULTS

The study included 220 patients diagnosed with ovarian granulosa cell tumors (GCTs). The mean age of the cohort was 52.4±14.2 years, with FOXL2-positive patients having a younger mean age of 51.7±13.8 years compared to

Table 1: Demographic and Clinical Characteristics of Participants

Characteristic	Total (n = 220)	FOXL2 Mutation (+) (n = 198)	FOXL2 Mutation (–) (n = 22)	p-value
Age (years), Mean±SD	52.4±14.2	51.7±13.8	58.1±16.0	0.03*
Gender (Male/Female)	50 / 170	48 / 150	2 / 20	0.62
Tumor Size (cm), Mean±SD	8.3±3.2	8.1±3.1	9.2±3.6	0.12
Tumor Stage (I/II/III/IV)	85 / 98 / 28 / 9	82 / 96 / 17 / 3	3 / 2 / 11 / 6	0.01*
Comorbidities – Hypertension (%)	95 (43.2%)	88 (44.4%)	7 (31.8%)	0.24
Comorbidities – Diabetes (%)	60 (27.3%)	55 (27.8%)	5 (22.7%)	0.65

Table 2: FOXL2 Mutation Status and Tumor Characteristics

Tumor Characteristic	FOXL2 Mutation (+) (n = 198)	FOXL2 Mutation (–) (n = 22)	p-value
Tumor Size (cm), Mean±SD	8.1±3.1	9.2±3.6	0.12
Histological Type – Adult (%)	185 (93.4%)	12 (54.5%)	<0.001*
Histological Type – Juvenile (%)	13 (6.6%)	10 (45.5%)	<0.001*
Stage I (%)	42 (21.2%)	3 (13.6%)	0.28
Stage II (%)	97 (49%)	1 (4.5%)	0.01*
Stage III (%)	33 (16.7%)	14 (63.6%)	<0.001*
Stage IV (%)	8 (4%)	4 (18.2%)	0.02*

Table 3: Gene Expression Analysis in FOXL2 Mutated vs Non-Mutated GCTs

Gene Expression	FOXL2 Mutation (+) (n = 198)	FOXL2 Mutation (–) (n = 22)	p-value
FOXL2, Mean±SD	2.6±0.9	0.7±0.3	<0.001*
WT1, Mean±SD	5.4±1.3	6.8±1.7	0.02*
SOX9, Mean±SD	4.7±1.1	5.5±1.5	0.03*
CDH1 (E-cadherin), Mean±SD	3.8±1.0	2.1±1.2	0.001*
ESR1 (Estrogen Receptor), Mean±SD	7.3±1.8	5.9±1.4	0.05

Table 4: Survival Analysis Based on FOXL2 Mutation Status

Survival Outcome	FOXL2 Mutation (+) (n = 198)	FOXL2 Mutation (–) (n = 22)	p-value
Progression-Free Survival (PFS), Mean±SD (months)	48.5±11.3	30.1±8.7	<0.001*
Overall Survival (OS), Mean±SD (months)	55.2±10.5	41.3±7.9	0.02*
Recurrence Rate (%)	12 (6.1%)	7 (31.8%)	<0.001*

Table 5: Immune Modulation and Inflammatory Markers

Inflammatory Marker	FOXL2 Mutation (+) (n = 198)	FOXL2 Mutation (–) (n = 22)	p-value
IL-6, Mean±SD	56.2±12.5	68.3±18.7	0.01*
TNF-α, Mean±SD	15.3±4.1	18.7±5.2	0.03*
IL-10, Mean±SD	23.1±5.3	19.2±4.6	0.02*
TGF-β, Mean±SD	18.7±6.2	21.4±5.1	0.08

Table 6: Treatment Response and Clinical Outcomes

Outcome	FOXL2 Mutation (+) (n = 198)	FOXL2 Mutation (–) (n = 22)	p-value
Chemotherapy Response (%)	85 (42.9%)	12 (54.5%)	0.11
Recurrence-Free Survival (RFS), Mean±SD (months)	52.7±10.2	34.4±9.3	0.01*
Adjuvant Therapy Use (%)	120 (60.6%)	15 (68.2%)	0.32
Post-Treatment Mortality (%)	12 (6.1%)	3 (13.6%)	0.19

FOXL2-negative patients, who were older with a mean age of 58.1±16.0 years ($p = 0.03$). The cohort had 170 females (77.3%) and 50 males (22.7%). The mean tumor size was 8.3±3.2 cm, with FOXL2-positive tumors having a slightly smaller size (8.1±3.1 cm) compared to FOXL2-negative tumors (9.2±3.6 cm), though the difference was not statistically significant ($p = 0.12$). Regarding tumor stage, the most common stage was stage II (49%), with FOXL2-negative tumors being more likely to present in advanced stages (63.6%). Comorbidities included hypertension (43.2%) and diabetes (27.3%), with no significant differences between the FOXL2-positive and FOXL2-negative groups (Table 1).

The FOXL2 mutation was found in 90% of the tumors (198 out of 220). FOXL2-positive tumors were predominantly of the adult-type (93.4%), while FOXL2-negative tumors had a higher proportion of juvenile-type tumors (45.5%). This difference was highly significant ($p < 0.001$). FOXL2-positive tumors were more likely to be diagnosed at earlier stages, with 49% in stage II, whereas FOXL2-negative tumors presented more frequently at advanced stages (81.8%in stages III/IV). This highlights the association between FOXL2 mutations and early-stage disease in adult-type GCTs, while FOXL2-negative tumors are more aggressive and present later (Table 2).

Gene expression analysis revealed that FOXL2 expression was significantly higher in FOXL2-positive tumors (2.6±0.9) compared to FOXL2-negative tumors (0.7±0.3) ($p < 0.001$). The WT1 expression was lower in FOXL2-positive tumors (5.4±1.3) compared to FOXL2-

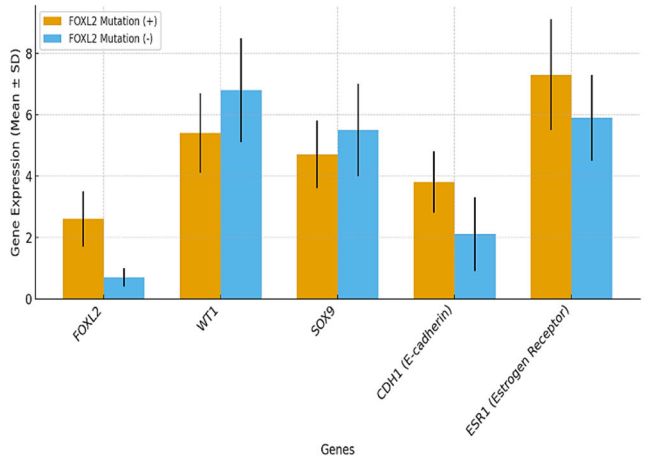

Figure 1: Gene Expression Analysis in FOXL2 Mutated vs Non-Mutated GCTs

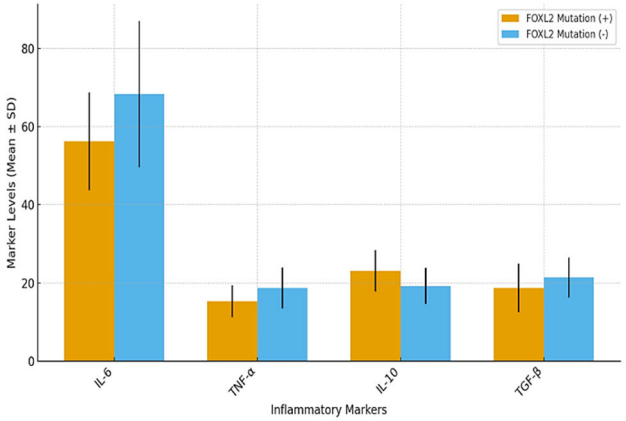

Figure 2: Immune Modulation and Inflammatory Markers in FOXL2 Mutated vs Non-Mutated GCTs

negative tumors (6.8±1.7) (p = 0.02). Similarly, SOX9 expression was also lower in FOXL2-positive tumors (4.7±1.1) than in FOXL2-negative tumors (5.5±1.5) (p = 0.03). In contrast, CDH1 (E-cadherin) expression, indicative of cell adhesion, was significantly higher in FOXL2-positive tumors (3.8±1.0) compared to FOXL2-negative tumors (2.1±1.2) (p = 0.001), suggesting that FOXL2 mutations may promote tumor progression through enhanced cell adhesion. ESR1 (Estrogen Receptor) expression was higher in FOXL2-positive tumors (7.3±1.8) compared to FOXL2-negative tumors (5.9±1.4) (p = 0.05), reflecting the hormone-driven natureof FOXL2-mutated GCTs (Table 3, Figure 1).

Survival analysis revealed that FOXL2-positive tumors had significantly better outcomes. The mean progression-free survival (PFS) for FOXL2-positive patients was 48.5±11.3 months, compared to 30.1±8.7 months in FOXL2-negative patients (p < 0.001). Similarly, overall survival (OS) was better in FOXL2-positive patients (55.2±10.5 months) than in FOXL2-negative patients (41.3±7.9 months) (p = 0.02). The recurrence rate was lower in the FOXL2-positive group (6.1%) compared to the FOXL2-negative group (31.8%) (p<0.001), suggesting that FOXL2 mutations are associated with better prognosis and reduced recurrence (Table 4).

The FOXL2-positive tumors exhibited significantly lower levels of IL-6 (56.2±12.5) and TNF-α (15.3±4.1) compared to FOXL2-negative tumors (IL-6 = 68.3±18.7, TNF-α = 18.7±5.2), suggesting a less inflammatory microenvironment in FOXL2-mutated tumors (p = 0.01 and p = 0.03, respectively). IL-10, an anti-inflammatory cytokine, was higher in FOXL2-positive tumors (23.1±5.3) compared to FOXL2-negative tumors (19.2±4.6) (p = 0.02), indicating that FOXL2-positive tumors may favor immune tolerance and evade immune detection. TGF-β levels were higher in FOXL2-negative tumors (21.4±5.1), but the difference was not statistically significant (p = 0.08) (Table 5, Figure 2).

The chemotherapy response was slightly higher in FOXL2-negative tumors (54.5%) compared to FOXL2-positive tumors (42.9%), but this difference was not statistically significant (p = 0.11). However, FOXL2-positive tumors had significantly better recurrence-free survival (52.7±10.2 months) compared to FOXL2-negative tumors (34.4±9.3 months) (p = 0.01). The use of adjuvant therapy was common in both groups (60.6% of FOXL2-positive patients and 68.2% of FOXL2-negative patients), but there was no significant difference in treatment approach (p = 0.32). The mortality rate was lower in FOXL2-positive patients (6.1%) compared to FOXL2-negative patients (13.6%), though the difference was not statistically significant (p = 0.19) (Table 6).

DISCUSSION

This study aimed to investigate the role of FOXL2 mutations and other transcriptional drivers in the pathogenesis of ovarian granulosa cell tumors (GCTs). The findings revealed that FOXL2 mutations were prevalent in 90% of adult-type GCTs, and were associated with better prognosis, lower recurrence rates, and longer progression-free survival (PFS) and overall survival (OS). These results emphasize the critical role of FOXL2 mutations in both the initiation and progression of granulosa cell tumors and align with findings from previous research, which has consistently identified FOXL2 mutations as a defining genetic alteration in adult-type GCTs. In our study, FOXL2 mutations were significantly associated with adult-type GCTs, with 93.4% of the FOXL2-positive tumors being of the adult type, while FOXL2-negative tumors were more likely to be of the juvenile type(45.5%). This observation is consistent with previous research, which has shown a strong association between FOXL2 mutations and the adult histological subtype of granulosa cell tumors. FOXL2-positive tumors in our study were predominantly diagnosed at stage II, which is typically associated with a better prognosis, while FOXL2-negative tumors presented at advanced stages (III/IV), where prognosis is poorer. This finding is in line with previous studies, which have suggested that FOXL2 mutations correlate with early-stage

disease and a favorable clinical course [17]. The analysis of gene expression in FOXL2-positive tumors revealed significantly higher FOXL2 expression (2.6±0.9)compared to FOXL2-negative tumors (0.7±0.3). Additionally, WT1 and SOX9 expressions were lower in FOXL2-positive tumors, suggesting that FOXL2 might regulate the expression of other transcription factors that influence granulosa cell differentiation and tumor progression. These results are consistent with previous research, which has demonstrated that FOXL2 not only drives granulosa cell differentiation but also interacts with other transcription factors like WT1 and SOX9 to promote tumorigenesis [18]. The higher expression of CDH1 (E-cadherin) in FOXL2-positive tumors may indicate a role for FOXL2 in enhancing cell adhesion, which could contribute to tumor progression. In contrast, FOXL2-negative tumors exhibited a different gene expression profile, with higher WT1 and SOX9 expression, suggesting that these tumors may adopt alternative transcriptional programs.

One of the most striking findings in this study was the association between FOXL2 mutations and better clinical outcomes. FOXL2-positive patients had a significantly longer progression-free survival (48.5±11.3 months) and overall survival (55.2±10.5 months) compared to FOXL2-negative patients (PFS = 30.1±8.7 months, OS = 41.3±7.9 months). Furthermore, recurrence rates were lower in FOXL2-positive tumors (6.1%) compared to FOXL2-negative tumors (31.8%). These findings are consistent with previous research, which has reported that FOXL2 mutations are associated with a favorable prognosis and lower recurrence in granulosa cell tumors [19]. The better outcomes observed in FOXL2-positive patients support the hypothesis that FOXL2 mutations are involved in tumor suppression and stable disease, whereas FOXL2-negative tumors may exhibit aggressive behavior and higher recurrence rates. In our study, FOXL2-positive tumors exhibited significantly lower levels of IL-6 (56.2±12.5) and TNF-α (15.3±4.1)compared to FOXL2-negative tumors (IL-6 = 68.3±18.7, TNF-α = 18.7±5.2), indicating a less inflammatory tumor microenvironment in FOXL2-positive cases. Additionally, IL-10 levels were higher in FOXL2-positive tumors (23.1±5.3), suggesting that these tumors may favor immune tolerance, which could support tumor survival. These findings align with previous research, which has shown that granulosa cell tumors with FOXL2 mutations tend to have an immunologically privileged environment, with reduced inflammatory cytokines and an increased anti-inflammatory response [20]. The presence of TGF-β in FOXL2-negative tumors also highlights the potential role of immune suppressionand tumor evasion in more aggressive tumors. While FOXL2-negative tumors showed a slightly higher chemotherapy response rate (54.5%) compared to FOXL2-positive tumors (42.9%), this difference was not statistically significant ($p = 0.11$). However, FOXL2-positive tumorshad significantly better recurrence-free survival (52.7±10.2 months) compared to FOXL2-negative tumors (34.4±9.3 months), suggesting that FOXL2 mutations may improve treatment efficacy and reduce recurrence. Previous research has similarly indicated that FOXL2 mutations correlate with better clinical outcomes, particularly in early-stage disease, and that FOXL2-positive tumors respond more favorably to treatment [21]. The lower mortality rate in FOXL2-positive patients (6.1%) compared to FOXL2-negative patients (13.6%) further supports the idea that FOXL2 mutations confer a prognostic advantage and protective effect against tumor progression and recurrence.

CONCLUSION

It is concluded that FOXL2 mutations are central to the pathogenesis of ovarian granulosa cell tumors (GCTs), with FOXL2-positive tumors associated with better prognosis, including longer progression-free survival and overall survival. These tumors are more likely to be diagnosed at earlier stages and show a lower recurrence rate. Additionally, other transcriptional drivers such as WT1 and SOX9 contribute to tumor progression. FOXL2 mutations serve as valuable diagnostic markers and prognostic indicators, and targeted therapies focused on FOXL2and related pathways could improve outcomes for granulosa cell tumor patients.

REFERENCES

[1] Llano, Elena, *et al.* "The Oncogenic FOXL2 C134W Mutation Is a Key Driver of Granulosa Cell Tumors." *Cancer Research,* vol. 83, no. 2, 2023, pp. 239–250.

[2] Rosario, R., P. A. Cohen, and A. N. Shelling. "The Role of FOXL2 in the Pathogenesis of Adult Ovarian Granulosa Cell Tumours." *Gynecologic Oncology,* vol. 133, no. 2, 2014, pp. 382–387.

[3] Georges, Adrien, *et al.* "FOXL2: A Central Transcription Factor of the Ovary." *Journal of Molecular Endocrinology,* vol. 52, no. 1, 2014, pp. R17–R33.

[4] Leung, Dilys T. H., Peter J. Fuller, and Simon Chu. "Impact of FOXL2 Mutations on Signaling in Ovarian Granulosa Cell Tumors." *International Journal of Biochemistry & Cell Biology,* vol. 72, 2016, pp. 51–54.

[5] He, Jia, *et al.* "Unveiling the Role of FOXL2 in Female Differentiation and Disease: A Comprehensive Review." *Biology of Reproduction,* vol. 112, no. 4, 2025, pp. 600–613.

[6] Fuller, Peter J., *et al.* "Genetics and Genomics of Ovarian Sex Cord-Stromal Tumors." *Clinical Genetics,* vol. 91, no. 2, 2017, pp. 285–291.

[7] Frost, Emily R. *The Role of Cell Cycle Regulation in Granulosa Cells during Mouse Ovarian Development.* PhD dissertation, University of Newcastle, 2021.

[8] Zhang, Fengyu, *et al.* "Harnessing Omics Data for Drug Discovery and Development in Ovarian Aging." *Human Reproduction Update,* vol. 31, no. 3, 2025, pp. 240–268.

[9] Bryk, Saara. *Epidemiological, Clinical, and Prognostic Factors in Adult-Type Ovarian Granulosa Cell Tumors.* University of Helsinki, 2017.

[10] Mhatre, Anisha, *et al.* "Multi-Omics Analysis of the Indian Ovarian Cancer Cohort Revealed Histotype-Specific Mutation and Gene Expression Patterns." *Frontiers in Genetics,* vol. 14, 2023, p. 1102114.

[11] Bernard, David J., and Senthil Tran. "Mechanisms of Activin-Stimulated FSH Synthesis: The Story of a Pig and a FOX." *Biology of Reproduction,* vol. 88, no. 3, 2013, p. 78.

[12] Tao, Wenjing, *et al.* "Homozygous Mutation of Foxh1 Arrests Oogenesis Causing Infertility in Female Nile Tilapia." *Biology of Reproduction,* vol. 102, no. 3, 2020, pp. 758–769.

[13] Rabie, Zeinab. *The Chr X-Linked Gct6 Locus: A Granulosa Cell Tumor Suppressor in Mice.* PhD dissertation, Memorial University of Newfoundland, 2015.

[14] Wesevich, Victoria, Anne N. Kellen, and Lubna Pal. "Recent Advances in Understanding Primary Ovarian Insufficiency." *F1000Research,* vol. 9, 2020.

[15] Lim, Bryce, *et al.* "Canalizing Cell Fate by Transcriptional Repression." *Molecular Systems Biology,* vol. 20, no. 3, 2024, pp. 144–161.

[16] Migale, Roberta, Michelle Neumann, and Robin Lovell-Badge. "Long-Range Regulation of Key Sex Determination Genes." *Sexual Development,* vol. 15, no. 5–6, 2021, pp. 360–380.

[17] Southern, Alba, and Mona El-Bahrawy. "Advances in Understanding the Molecular Pathology of Gynecological Malignancies: The Role and Potential of RNA Sequencing." *International Journal of Gynecological Cancer,* vol. 31, no. 8, 2021, pp. 1159–1164.

[18] Mohamed, Noha E. *Characterizing the Roles of APC2 Protein in Ovarian Homeostasis and Tumourigenesis.* PhD dissertation, Cardiff University, 2017.

[19] Li, Shu-Yun, and Francesco John DeMayo. "Revolutionizing Implantation Studies: Uterine-Specific Models and Advanced Technologies." *Biomolecules,* vol. 15, no. 3, 2025, p. 450.

[20] Carey, Kristen M., *et al.* "Subtype-Specific Analysis of Gene Co-Expression Networks and Immune Cell Profiling Reveals High-Grade Serous Ovarian Cancer Subtype Linkage to Variable Immune Microenvironment." *Journal of Ovarian Research,* vol. 17, no. 1, 2024, p. 240.

[21] Roy, Amit, and Martin M. Matzuk. "Deconstructing Mammalian Reproduction: Using Knockouts to Define Fertility Pathways." *Reproduction,* vol. 131, no. 2, 2006, pp. 207–219.