GYNAECOLOGICAL PATHOLOGY

Ovarian sex cord-stromal tumours: an update in recent molecular advances

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Summary

Sex cord-stromal tumours (SCSTs) consist of a heterogeneous group of neoplasms with diverse clinicopathological features and biological behaviour. They often present as a diagnostic challenge as they have varied and occasionally overlapping histomorphology and some may even mimic non-SCSTs. An accurate diagnosis is important for therapeutic and prognostic purposes. The use of a panel of immunohistochemical markers which are sensitive and specific for sex cord-stromal differentiation such as α -inhibin, calretinin, SF-1 and FOXL2, may be helpful in confirming the cellular lineage of these tumours, but is of limited utility in distinguishing between the different tumour types within this category. Additionally, the development of new therapeutic strategies in patients with SCSTs is also hampered by the infrequent occurrence of these neoplasms.

Recent molecular analyses of some SCSTs has led to the discovery of novel molecular events, which may have important diagnostic, prognostic and therapeutic implications. The salient pathological features, management issues and recently described genetic aberrations in adult and juvenile granulosa cell tumours as well as Sertoli–Leydig cell tumours are discussed in this review, with particular emphasis on the clinical significance of *FOXL2* and *DICER1* mutations. An in-depth understanding of the molecular pathogenesis underlying SCSTs may aid in improving tumour classification and disease prognostication and also potentially lead to the discovery of more effective treatment strategies.

Key words: Sex cord-stromal; FOXL2; DICER1; immunohistochemistry; molecular; ovary.

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INTRODUCTION

Sex cord-stromal tumours (SCSTs) are a diverse group of neoplasms derived from either gonadal sex cord cells (granulosa and sertoli cells), gonadal stromal cells (theca cells), or fibroblasts.¹ They account for 8% of all primary ovarian tumours and encompass entities with widely varied clinicopathological features and biological behaviour. In the most recent World Health Organization (WHO) classification of tumours of the female reproductive organs, SCSTs are

separated into pure stromal, pure sex cord and mixed SCSTs² (Table 1). Whilst most tumours have characteristic morphological appearance, some can exhibit unusual or overlapping features and even mimic non-SCSTs. In these instances, ancillary techniques, such as immunohistochemistry may be useful in confirming the cellular lineage of these tumours. Most SCSTs express α -inhibin, calretinin and steroidogenic factor-1 (SF-1) and are typically negative for epithelial and germ cell tumour markers. More recently, FOXL2 (Foxhead box protein L2) has also been identified to be a sensitive and specific marker of sex cord-stromal differentiation. However, no immunomarker shows absolute sensitivity or specificity for SCSTs and most have limited utility in distinguishing between the different tumour types within this category.

Recent studies have led to the discovery of novel molecular events, which may have important diagnostic, prognostic and therapeutic implications in this group of tumours. In this review, we discuss the salient pathological features, management issues and recently described genetic aberrations in granulosa cell and Sertoli–Leydig cell tumours, and examine the clinical significance of these molecular findings.

GRANULOSA CELL TUMOURS

Granulosa cell tumours represent 90% of all ovarian SCSTs and constitute ~5% of ovarian cancers. They are divided into two categories: adult GCTs (AGCTs) and juvenile GCTs (JGCTs) on the basis of differing clinicopathological characteristics. Evidence provided by various molecular studies also indicates that these tumours are distinct entities.

Adult granulosa cell tumours

AGCTs constitute 95% of all granulosa cell tumours and are the most common malignant SCSTs.^{3,4} They can occur at any age but are commonly seen in perimenopausal or early postmenopausal women with a peak age incidence between 50 and 55 years.⁵ Patients often present with oestrogenic manifestations such as menometrorrhagia or post-menopausal bleeding and some may have underlying endometrial hyperplasia and/or adenocarcinoma. Rarely, AGCT can be associated with androgenic manifestations, especially if they are cystic.⁶ Patients may also present with abdominal pain and distension secondary to a large pelvic mass.

The majority (90%) of tumours are unilateral with an average diameter of 12 cm, although size may vary from microscopic to ~30 cm. Cut surface typically reveals a tan (Fig. 1A) to yellowish solid or solid-cystic appearance, but

Table 1 WHO classification of ovarian sex cord-stromal tumours

Pure stromal tumours
Fibroma
Cellular fibroma
Thecoma
Luteinised thecoma associated with sclerosing peritonitis
Fibrosarcoma
Sclerosing stromal tumour
Signet-ring stromal tumour
Microcystic stromal tumour
Leydig cell tumour
Steroid cell tumour
Steroid cell tumour, malignant
Pure sex cord tumours
Adult granulosa cell tumour
Juvenile granulosa cell tumour
Sertoli cell tumour
Sex cord tumour with annular tubules
Mixed sex cord-stromal tumours
Sertoli–Leydig tumours
Well differentiated
Moderately differentiated
With heterologous elements
Poorly differentiated
With heterologous elements
Retiform
With heterologous elements
Sex cord tumour, NOS

NOS, not otherwise specified; WHO, World Health Organization.

rarely, tumours may be predominantly cystic. On microscopic examination, AGCTs can exhibit a wide spectrum of morphological features, with a combination of growth patterns often encountered within the same tumour. The granulosa cells have ovoid or round nuclei, pale chromatin and often, nuclear grooves, imparting a ‘coffee-bean’ appearance (Fig. 1B). The cytoplasm is usually scant, although luteinised cells with moderate to abundant eosinophilic cytoplasm, round to ovoid nuclei with prominent nucleoli and lacking nuclear grooves may be seen.^{7,8} Mitotic rate is variable but generally low (<5/10 HPF). Bizarre cells may be identified in ~2% of AGCTs⁹ and rare tumours with sarcomatous transformation¹⁰ or hepatic differentiation¹¹ have been reported. Cells can be arranged in a variety of architectural patterns including diffuse or sheet-like growth, microfollicular, macrofollicular, trabecular, insular, tubular (solid or hollow), corded, watered silk and gyriform (zigzag cords). Call–Exner bodies (Fig. 1C) are a feature of the microfollicular pattern, and are characterised by microcystic spaces containing eosinophilic basal lamina material. The presence of nuclear

grooves and Call–Exner bodies are often considered to be pathognomonic of AGCTs, however these features may be lacking in some tumours and their absence does not preclude a diagnosis of AGCT.

Chromosomal aberrations

Using cytogenetic¹² and comparative genomic hybridisation analyses,¹³ trisomy of chromosomes 12 and 14 and monosomy of chromosome 22 have been detected in a subset of AGCTs.^{12,13} Caburet *et al.* also reported the presence of supernumerary chromosomes 8, 9, 12 and 14 as well as partial or complete loss of chromosomes 1p, 13p, 16, 11 and 22, with a statistically significant non-random association for +14 with –22 and +7 with –16q.¹⁴

FOXL2

In a landmark study using whole-transcriptome sequencing, Shah *et al.* identified a recurrent, somatic missense mutation in codon 134 (402C→G) of *FOXL2* (Fig. 2) in four AGCTs.¹⁵ This mutation was also identified in 86/89 (97%) additional AGCTs, but only in 3/14 (21%) thecomas and 1/10 (10%) JGCTs, and none of the 49 ovarian SCSTs of other types, 149 epithelial ovarian tumours and 180 breast carcinomas. It has since been postulated that this mutation is the driver event in AGCTs.¹⁵

FOXL2 is a single-exon gene of 2.7 kb located at 3q23 that encodes for a 376 amino acid protein, which is a member of the forkhead–winged helix family of transcription factors containing a highly conserved DNA-binding forkhead domain. *FOXL2* is selectively expressed in the mesenchyme of developing mouse eyelids and in the adult ovarian follicle. It is one of the earliest markers of ovarian differentiation, and is essential for the normal development of granulosa cells.^{16,17} *FOXL2* has also been linked to the regulation of several key biological processes, such as apoptosis, oxidative stress response and cell proliferation. Several targets of *FOXL2* have been identified in gonadal cells¹⁸ including genes fundamental to granulosa cell function and the development of ovarian follicles.¹⁹ Regulation of gene expression by *FOXL2* involves interactions with other transcription factors such as nuclear receptors and the SMAD family of transcription factors.^{20,21}

Germline mutations in *FOXL2* result in the blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES), either with or without associated premature ovarian failure.²² The somatic missense point mutation (C402G)

Fig. 1 Adult granulosa cell tumour. (A) The tumour is solid with a tan cut surface. (B) The cells have ovoid nuclei and longitudinal nuclear grooves. (C) Call–Exner bodies are noted.

associated with AGCTs, leads to the substitution of a highly conserved cysteine residue by tryptophan (C134W), although this does not appear to affect either function or localisation of the protein.¹⁵ This mutation is seen in the heterozygous state in most tumours, however the exact mechanism by which the C134W mutation in *FOXL2* contributes to tumourigenesis of granulosa cells remain unresolved. *In vitro* studies suggest that dysregulated *FOXL2* function may alter cell cycle progression, steroidogenesis, apoptosis and even DNA damage repair in granulosa cells.^{23–25} Gene expression studies have also shown that AGCTs are significantly enriched for known *FOXL2* target genes, affirming the importance of this mutation in these tumours.²⁶

Using single-strand conformation polymorphism analysis, Kim *et al.* showed that 53/56 (94.6%) AGCTs harboured the *FOXL2* p.C134W mutation.²⁷ Similarly, other investigators have identified *FOXL2* mutations in 61–95% of ovarian AGCTs.^{27–36} Nolan *et al.* also identified *FOXL2* mutations in 6/12 granulosa theca cell tumours (ovarian SCSTs that have between 10% and 50% of granulosa cells in a prominent fibrothecomatous background), and the authors noted that tumours with higher content of granulosa cells as well as those in which the granulosa cells formed large lobules were more likely to harbour the mutation.³⁷ Interestingly, a small number of testicular AGCTs have also been found to harbour this aberration. Lima *et al.* identified *FOXL2* mutation in 2/5 (40%) AGCTs but not in nine other testicular tumours (1 JGCT, 5 Leydig cell tumours and 3 SLCTs).³⁸

In addition to AGCTs, *FOXL2* mutations have been reported in only 7/45 (15%) thecomas, 2/59 (3.4%) JGCTs and 5/63 (8%) SLCTs.^{15,27,29,39} This mutation has not been demonstrated in other ovarian SCSTs such as fibromas, gynandroblastomas, sex-cord tumours with annular tubules, sclerosing stromal tumours, microcystic stromal tumours, steroid cell tumours; germ cell tumours, epithelial or mesenchymal tumours.^{15,27–31,33,34,39–44} Table 2 summarises the results from various studies evaluating *FOXL2* mutation status in different subtypes of ovarian tumours. Of note, some thecomas harbouring the mutation were reclassified as AGCTs on review,⁴⁵ while reappraisal of some AGCTs lacking the mutation suggests that they may have been misclassified initially.³⁰ However, the presence of *bona fide* AGCTs lacking *FOXL2*

mutations, albeit rare, also indicates that it is plausible that other molecular events can result in a morphological AGCT phenotype.¹⁵

Overall, it appears that *FOXL2* mutation is a sensitive and specific biomarker for AGCTs which can be employed in a diagnostic setting to help resolve problematic cases. Additionally, one group of investigators was able to detect the *FOXL2* mutation in circulating tumour DNA from a subset of patients with AGCTs, demonstrating that a molecular diagnosis of AGCTs and detection of tumour recurrences can also be achieved through non-invasive methods.⁴⁶

Immunohistochemical profile

Alpha-inhibin, calretinin (Fig. 3) and SF-1 are sensitive markers of AGCTs.^{47–53} Compared to α -inhibin, calretinin is a more sensitive but less specific marker of sex cord-stromal differentiation.⁵¹ SF-1 is expressed in both adult and juvenile granulosa cell tumours and studies have reported that the sensitivity of SF-1 for granulosa cell tumours is 100%.^{47,53} However, SF-1 can also be expressed by other SCSTs,^{53,54} limiting its use in distinguishing AGCTs from other tumour types in this category. Additionally, AGCTs can exhibit immunoreactivity for vimentin, CD99 and WT-1.^{55,56} In comparison to SF-1, WT-1 is less sensitive and specific for SCSTs and it is also less sensitive for granulosa cell tumours.^{53,55}

AGCTs can display variable positivity for CD56, AE1/3, CAM5.2, smooth muscle actin, desmin, S100, ER and PR but typically do not express CD34, CK7 or EMA.^{48,57–61} Areas of hepatic differentiation may be AFP, CEA, EMA and CAM5.2 positive but are vimentin and α -inhibin negative.⁶²

FOXL2 is a recently described immunomarker expressed in almost all adult and juvenile granulosa cell tumours.^{27,28,39}

However, in contrast to the specificity of *FOXL2* mutations, nuclear *FOXL2* immunoexpression is seen in almost all ovarian SCSTs, including those lacking the corresponding mutation.^{27,39} In one study, *FOXL2* immunoexpression was identified in 95/119 (80%) SCSTs, including: 41/42 (97.6%) AGCTs, 20/40 (50%) SLCTs, 9/9 JGCTs, 9/9 fibromas, 5/5 sclerosing stromal tumours, 3/5 (60%) thecomas, 3/3 fibrosarcomas, 2/2 sex-cord tumours with annular tubules, 1/11 (9%) steroid cell tumour and 3/4 (75%) unclassified sex cord tumours. No *FOXL2* immunoreactivity was detected in: Leydig cells of SLCTs, all nine retiform SLCT, all six Leydig cell tumours and all four stromal luteomas. Among all other non-SLCTs tested ($n = 368$), only three female adnexal tumours of probable Wolffian origin (FATWO) were positive for this marker. In contrast, only 38% of the SCSTs harboured *FOXL2* mutations, with AGCTs accounting for the vast majority (39/42, 93%) of them. All except for one *FOXL2* mutant tumour exhibited *FOXL2* immunoreactivity. None of the non-SCST cases tested, including the three FATWOs, harboured a *FOXL2* mutation.³⁹ In this study, the authors also compared the immunoexpression of *FOXL2*, α -inhibin and calretinin, in a series of 89 SCSTs.³⁹ It was shown that *FOXL2* was a more sensitive marker for SCSTs than α -inhibin and calretinin, and immunoreactive tumours demonstrated a stronger staining pattern with *FOXL2* than either α -inhibin or calretinin. Retained immunoexpression for α -inhibin and/or calretinin was noted in SLCTs lacking *FOXL2* staining. In another study, Kim *et al.*²⁷ evaluated the expression of α -inhibin and *FOXL2* in 56 AGCTs, five

Fig. 2 Adult granulosa cell tumour with c.402C>G mutation of the *FOXL2* gene (arrow). (Figure courtesy of Dr Britta Weigelt.)

Table 2 *FOXL2* mutation in ovarian and non-ovarian tumours

Reference	Tumours with <i>FOXL2</i> mutation
Shah <i>et al.</i> , 2009 ¹⁵	90/93 (97%) AGCTs 1/10 (10%) JGCTs 3/14 (21%) Thecomas 0/24 (0%) Fibromas 0/10 (0%) Steroid cells tumours
Schrader <i>et al.</i> , 2009 ⁴⁴	13/13 (100%) AGCTs 0/725 (0%) Epithelial and mesenchymal tumours
Kim <i>et al.</i> , 2010 ²⁷	53/56 (95%) AGCTs 0/6 (0%) JGCTs 2/16 (12.5%) Thecomas 0/3 (0%) Fibrothecomas 0/4 (0%) SLCTs 0/1 (0%) SCTAT 0/4 (0%) SSTs 0/85 (0%) Ovarian epithelial carcinomas 0/1167 (0%) Non-ovarian malignancies
Kim <i>et al.</i> , 2010 ³¹	18/20 (90%) AGCTs 0/3 (0%) JGCTs
Jamieson <i>et al.</i> , 2010 ³⁰	52/56 (93%) AGCTs 0/3 (0%) JGCTs 0/3 (0%) Ovarian carcinomas
D'Angelo <i>et al.</i> , 2011 ²⁸	19/27 (70%) AGCTs 0/18 (0%) JGCTs
Al-Agha <i>et al.</i> , 2011 ³⁹	39/42 (93%) AGCTs 0/9 (0%) JGCTs 0/9 (0%) Fibromas 2/5 (40%) Thecomas 0/5 (0%) SSTs 3/40 (7%) SLCTs 1/4 (25%) SCSTs, unclassified 0/2 (0%) SCTATs 0/11 (0%) Steroid cell tumours 0/6 (0%) Ovarian germ cell tumours 0/322 (0%) Ovarian epithelial tumours 0/7 (0%) Mesenchymal tumours 0/1 (0%) Small cell carcinoma, hypercalcaemic type 0/3 (0%) FATWO 0/30 (0%) Metastatic epithelial tumours
Oparka <i>et al.</i> , 2012 ³³	4/5 (80%) AGCTs 0/5 (0%) Fibromas 0/6 (0%) Gynandroblastomas
Rosario <i>et al.</i> , 2013 ³⁵	18/26 (69%) AGCTs
McCluggage <i>et al.</i> , 2013 ⁴²	0/22 (0%) Cellular fibromas
Kommos <i>et al.</i> , 2014 ³²	40/41 (98%) AGCTs
Oseto <i>et al.</i> , 2014 ³⁴	27/44 (61%) AGCTs 0/2 (0%) JGCTs 0/3 (0%) Germ cell tumours 0/63 (0%) Surface epithelial-stromal tumours
Irving <i>et al.</i> , 2015 ⁴¹	0/14 (0%) Microcystic stromal tumours
Zannoni <i>et al.</i> , 2016 ³⁶	33/37 (89%) AGCTs
Goulvent <i>et al.</i> , 2016 ²⁹	95/101 (94%) AGCTs 1/8 (13%) JGCTs 2/19 (11%) SLCTs 0/10 (0%) Fibrothecomas 0/2 (0%) Sertoli cell tumours 0/2 (0%) Steroid cell tumours 0/1 (0%) SST 0/1 (0%) SCTAT 0/12 (0%) Undifferentiated SCSTs 0/3 (0%) Microcystic stromal tumours
Meurgey <i>et al.</i> , 2017 ⁴³	31/35 (89%) AGCTs
Buza <i>et al.</i> , 2017 ⁴⁰	1/4 (25%) SLCTs 1/11 (9%) Fibromas/Fibrothecomas

AGCT, adult granulosa cell tumour; FATWO, female adnexal tumour of probable Wolffian origin; JGCT, juvenile granulosa cell tumour; SCST, sex cord-stromal tumour; SCTAT, sex cord tumour with annular tubules; SLCT, Sertoli–Leydig cell tumour; SST, sclerosing stromal tumour.

JGCTs, and two thecomas. Alpha-inhibin was expressed in 50/56 (89%) AGCTs, 4/5 (80%) JGCTs and both thecomas, while all the tumours in this cohort stained for FOXL2, regardless of their *FOXL2* mutation status. FOXL2 immunoeexpression has also been described in microcystic stromal tumours,⁴³ which lack *FOXL2* mutations.^{41,43}

Overall, evidence indicates that while FOXL2 has limited value in differentiating between different subtypes of SLCTs, it is a relatively sensitive and highly specific marker for SCSTs and together with α -inhibin and calretinin, constitutes an immunomarker panel that will allow for the identification of most SCSTs. A tumour lacking FOXL2, α -inhibin and calretinin expression is highly unlikely to be a SCST and the possibility of other tumour types should be excluded.³⁹

Management and outcome

Most patients with AGCTs present with disease confined to the ovary, although all AGCTs have a malignant potential and may develop pelvic/lower abdominal recurrences or less commonly, distant metastases. Recurrences have been reported up to 30 years after initial surgery, underpinning the importance of long-term follow-up.⁶³ Stage is the most important prognostic factor.^{64,65} Patients with stage I tumours have a much better 10-year survival rate than those with advanced stage disease (86% versus 49%);⁶⁶ however, the 25-year survival rate is considerably lower (40–60%), due to late tumour recurrences.⁶⁷

One of the major challenges in the management of patients with AGCTs lies in the identification of those at risk of developing disease recurrences. Various clinicopathological and molecular parameters such as age, tumour size (>5 cm), tumour rupture, incomplete tumour debulking, nuclear atypia, mitotic rate, diffuse growth, reduced β -catenin expression, expression of transcription factor GATA4 and human epidermal growth factor receptor HER2 have been reported to be associated with worse outcome in patients with AGCTs.^{64,67–77} However, none of these parameters have been conclusively proven to reliably predict for disease recurrence. More recently, D'Angelo *et al.* reported that patients with *FOXL2* mutated tumours had a worse disease-free and overall survival than those with the wild-type gene²⁸ while Rosario *et al.* showed that women with homozygous *FOXL2* mutations had significantly higher relapse rates.³⁵ These findings remain to be further validated. Similarly, although granulosa theca cell tumours can also harbour the same *FOXL2* mutation that characterises AGCT, there is currently no evidence to suggest that this should alter the classification or management of these neoplasms.³⁷

Total hysterectomy with bilateral salpingo-oophorectomy is the standard therapy in peri- or post-menopausal women with AGCT. For those of child-bearing age, appropriate counselling and fertility-sparing surgical options may be considered in stage 1A disease. In these instances, endometrial assessment should also be performed pre-operatively to exclude the possibility of co-existing endometrial hyperplasia and/or adenocarcinoma.⁷⁸ The main challenge in the treatment of AGCTs lies in the management of recurrent or advanced stage disease. Currently, patients may be offered secondary cytoreductive surgery, chemotherapy or hormonal therapy (including aromatase inhibitors) and/or radiotherapy.^{79–82} However, these modalities often demonstrate only limited efficacy. The role of systemic therapy following

Fig. 3 Adult granulosa cell tumour demonstrating patchy nuclear and cytoplasmic calretinin positivity.

complete surgical debulking is also controversial. Close clinical up-follow or endocrine therapy has been adopted in some patients.⁸³ The use of anti-angiogenic agents has also been explored, but the overall efficacy of these drugs remains to be assessed.⁸⁴

Juvenile granulosa cell tumour

JGCTs account for 5–15% of all granulosa cell tumours and occur most commonly within the first three decades of life.⁸⁵ Prepubertal patients often present with isosexual pseudoprecocity secondary to hyperoestrogenism,⁸⁶ although rarely, some may exhibit features of virilisation.⁸⁷ Abdominal pain, distension and menstrual irregularities are common presentations in post-pubertal women.⁸⁵

The vast majority of JGCTs present as unilateral tumours ranging in size from 3 to >30 cm. Most neoplasms have solid and cystic cut surfaces but some may be entirely solid or cystic. The solid areas show a tan-yellow or greyish appearance with foci of haemorrhage and/or necrosis while the cysts have serous or haemorrhagic contents.⁸⁵ Microscopic examination reveals a nodular (Fig. 4A) or diffuse proliferation of cells embedded in an oedematous or myxoid stroma. The presence of follicle-like spaces of varying sizes and shapes, containing eosinophilic or basophilic secretions, is a characteristic feature. Cells show round, hyperchromatic nuclei and abundant eosinophilic or vacuolated cytoplasm (Fig. 4B). A variable degree of cytological atypia, including the presence of bizarre cells, may be encountered (Fig. 4C). Mitotic activity is also variable but generally brisk and atypical mitotic figures can be seen.

Immunohistochemistry

Tumour cells are positive for α -inhibin,^{52,88} calretinin,^{48,51} SF-1 and often express vimentin,⁸⁸ WT-1,⁴⁸ CD56,⁶⁰ CD99,⁵⁶ CD10,⁸⁹ SMA,⁹⁰ S100, cytokeratin⁸⁸ and rarely, EMA.⁴⁸ FOXL2 immunoexpression has also been identified in JGCTs (Fig. 4D).

Genetic susceptibility

Most JGCTs occur sporadically, but some can arise in association with Ollier's disease (enchondromatosis)^{85,91,92} and Maffucci's syndrome (enchondromatosis and soft tissue haemangiomas).⁹³ Patients with Ollier's disease have an increased risk of developing central chondrosarcoma,

gliomas and non-small cell lung carcinoma,^{94–96} while those with Maffucci's syndrome are predisposed to astrocytomas, pituitary adenomas and pancreatic adenocarcinomas.^{95,97}

Molecular profile

Unlike AGCTs, the molecular basis of JGCTs has been less well characterised. In contrast to AGCTs, *FOXL2* mutations rarely occur in JGCTs. Instead two activating mutations of the stimulatory α subunit of a trimeric G protein (*G α s*), located at position 201, have been described in 30% of JGCT in one cohort.⁹⁸ These R201C and R201H mutations may inhibit GTPase activity, maintaining *G α s* in its active form, thus predisposing to cellular proliferation and tumour invasion.^{99,100} The same investigators also detected in-frame tandem duplications of exon 3 of *AKT1* as well as an array of *AKT1* point mutations resulting in the alteration of highly conserved residues, in a series of 16 JGCTs.¹⁰¹ Further analysis of the RNA-Seq data revealed a series of differentially expressed genes involved in cytokine and hormone signalling and cell division-related processes, suggesting that *ATK1* activation may play a crucial role in the pathogenesis of JGCTs.^{101,102}

Somatic *DICER1* mutations have also been identified in JGCTs. In one study, one of 14 JGCTs was found to harbour a somatic 'hotspot' *DICER1* mutation,¹⁰³ but none were identified in four JGCTs in another study.¹⁰⁴

It has been suggested that the JGCTs occurring in patients with Ollier's disease and Maffucci's syndrome may have underlying somatic mosaic *IDH1* and *IDH2* mutations (predominantly involving Arg132 of *IDH1* or Arg172 of *IDH2*), since these molecular aberrations have been described in both conditions.¹⁰⁵ Interestingly, Kenny *et al.* reported an ovarian cellular fibroma occurring in a patient with Ollier's disease, which also harboured the *IDH1* R123 mutation.¹⁰⁶ The mutant *IDH* gene produces 2-hydroxyglutarate (2-HG) which induces histone hypermethylation.¹⁰⁷ However, the exact role of either the mutant *IDH* gene or 2-HG in the pathogenesis of JGCT remains uncertain.¹⁰⁸

Management and outcome

The prognosis of patients with JGCT is stage dependent. It has been suggested that the presence of gsp mutation may also have prognostic significance as patients with tumours harbouring this mutation often present with advanced stage disease or tumour recurrence.⁹⁸ Similarly, another group of investigators also reported that patients with tumours demonstrating strong FOXL2 protein expression had worse overall and disease-free survival compared those with negative or weakly immunoreactive tumours,²⁸ however this observation remains to be validated with further studies.

The survival rate for patients with stage I tumours is approximately 97%, however those with advanced stage tumours usually succumb to the disease.⁸⁵ The presence of cytological atypia or brisk mitotic activity does not affect prognosis in stage I tumours.⁸⁸

Unilateral salpingo-oophorectomy with staging should be considered in young patients with unilateral disease wishing to preserve fertility, whereas total hysterectomy with bilateral salpingo-oophorectomy is the treatment of choice in patients with advanced stage disease.¹⁰⁹ Adjuvant chemotherapy is also a consideration in this latter group as well as in patients

with recurrent disease.¹⁰⁹ In comparison to AGCTs, JGCTs are less likely to recur or metastasise and any tumour recurrences usually occur earlier (within 3 years of diagnosis).⁸⁵

SERTOLI–LEYDIG CELL TUMOURS

SLCTs are rare neoplasms constituting <0.5% of all ovarian tumours. They occur in patients of all age groups (mean 25 years) with well-differentiated tumours occurring in slightly older (mean 35 years) and retiform variants in younger women (mean 15 years). Patients may present with symptoms related to an ovarian mass, virilisation or less commonly, hyperoestrogenic manifestations. The current WHO classification categorises SLCTs into well, moderate and poorly differentiated and retiform variants. Moderate and poorly differentiated as well as retiform variants may contain heterologous elements.¹¹⁰

Most tumours are unilateral and can vary in size from microscopic to >20 cm, with larger size being a more common feature of poorly differentiated neoplasms.¹¹¹ Gross examination shows solid, yellowish, lobulated cut surface although a cystic appearance can be appreciated in tumours with retiform or heterologous elements.^{112,113} The cysts in retiform neoplasms may contain oedematous or gelatinous, papillary or polypoid projections. Foci of necrosis and haemorrhage are common in poorly differentiated SLCTs.

On microscopic examination, well-differentiated SLCTs tend to be circumscribed and are composed of lobules of solid or hollow tubules, separated by fibromatous stroma. The tubules are lined by cuboidal to columnar Sertoli cells with basally placed nuclei, small nucleoli and abundant eosinophilic or pale vacuolated cytoplasm. Leydig cells are present within the stroma and some may also line tubules. The Leydig cells, which are seen singly or in groups, have

variable intracytoplasmic lipid contents and some may also contain lipochrome pigment or Reinke crystals.¹¹⁴ Cytological atypia and mitotic activity are rare.

A lobular architecture is also seen in moderately differentiated SLCTs, with cellular areas separated by oedematous or less frequently fibromatous stroma. The cellular areas are composed of Sertoli cells arranged in nests, cords, solid or poorly formed tubules or diffuse sheets. Infrequently, cysts containing eosinophilic secretions as well as follicle-like spaces may be seen. The Sertoli cells appear immature with high nucleo-cytoplasmic ratio, small round to oval nuclei and inconspicuous nucleoli. Leydig cells may be present at the periphery of the lobules of Sertoli cells or more commonly, within the oedematous stroma (Fig. 5A).

Poorly differentiated SLCTs are characterised by a diffuse or spindle (sarcomatoid) proliferation of poorly differentiated Sertoli cells with hyperchromatic nuclei and scant cytoplasm, often associated with brisk mitotic activity (>10/10 HPFs). Leydig cells are typically inconspicuous.¹¹⁴ The histological appearance may be indistinguishable from that of a pure sarcoma, with more characteristic Sertoli cell elements and Leydig cells identified only after extensive sampling.

A retiform component is present in 15% of SLCTs.^{114–116} Microscopically, this is characterised by a network of irregularly branching, narrow slit-like tubules and cysts with papillary or polypoid projections¹¹⁵ (Fig. 5B). The cysts and tubules may contain eosinophilic secretions and are lined by cuboidal cells with scant cytoplasm and round to oval nuclei, often exhibiting varying degrees of cytological atypia. Papillary and polypoid formations may be: (1) small and round or blunt with hyalinised cores (most common); (2) large and bulbous with oedematous cores; or (3) delicate and branching and lined by stratified cells reminiscent of the

Fig. 4 Juvenile granulosa cell tumour. (A) Low magnification view showing a lobulated arrangement of tumour cells with intervening collagenous and oedematous stroma. (B) Granulosa cells with round, non-grooved nuclei and abundant vacuolated or eosinophilic cytoplasm are embedded in a myxoid stroma. (C) Tumour cells exhibiting marked cytological atypia. (D) Tumour cells showing FOXL2 nuclear expression. (Figure courtesy of Dr Robert Soslow.)

papillary projections seen in serous borderline tumours. The surrounding stroma can be oedematous, hyalinised or densely cellular and immature.¹¹⁷

Heterologous elements are found in 20% of SLCTs, most frequently in moderately or poorly differentiated tumours and also in the retiform variants.¹¹¹ The most common heterologous element is the intestinal-type mucinous epithelium^{112,118} (Fig. 5C). This usually has a benign morphology although features of a borderline or malignant tumour have been described.¹¹³ Less commonly encountered heterologous elements include rhabdomyoblasts (Fig. 5D),¹¹¹ cartilage,¹¹² hepatoid elements,¹¹⁹ carcinoid tumour,¹¹² or neuroblastoma.¹¹¹

Immunohistochemical profile

SLCTs commonly express α -inhibin, calretinin (more intense staining in Leydig cells), SF-1 and WT-1^{39,53,54,120–122} and can also demonstrate immunoreactivity for CD99,^{48,53,56} Melan-A,¹²³ CD56, CD10⁸⁹ and cytokeratin.⁴⁸ FOXL2 is expressed in ~50% of SLCTs and immunoreactivity is confined to the Sertoli cells and in non-retiform variants.³⁹ Poorly differentiated SLCTs may show minimal or no expression for markers of sex cord differentiation. EMA is also typically negative, except in heterologous epithelial elements.⁴⁸

Molecular profile

Somatic *DICER1* mutations have been identified in ~60% of SLCTs.^{103,124–127} *DICER1* encodes for a RNA endonuclease that helps cleave precursor miRNA into mature miRNAs.¹²⁸ MiRNAs are small (20–22 nucleotide) non-coding RNAs that regulate the translation of downstream

target genes, with a crucial role in virtually all biological processes. Mutation in any component of the miRNA processing pathway can affect miRNAs expression, resulting in dysregulation of their target mRNA expression. MiRNAs are usually downregulated in cancer¹²⁹ and have been shown to have oncogenic or tumour suppressive functions. The expression levels of *DICER1* have widespread effects on the biogenesis of miRNA, and reduced expression has been associated with poor outcome in different cancer types.^{130–134} Studies using mouse models have shown that loss of a single *DICER1* allele (haploinsufficiency) reduces the time to tumour formation¹³⁵ and is also associated with poorer survival.¹³⁶

DICER1 mutations in SLCTs are missense ‘hotspots’ mutations involving codons encoding metal-binding sites within the RNase IIIb catalytic centres, which are essential for microRNA interaction and cleavage.¹⁰³ In one study, 38% of SLCTs were found to harbour the p.D1709N (c.5125G>A) mutation in *DICER1*¹⁰³ while another study reported the presence of p.E1705K (c.5113G>A) in 80% of *DICER1*-mutant tumours¹²⁴ (Fig. 6). There is no apparent association between *DICER1* mutation status and the presence of heterologous or retiform differentiation. Interestingly, well-differentiated SLCTs do not appear to be associated with *DICER1* mutations, however the number of tumours tested is limited and this finding remains to be validated with a larger cohort.^{29,124}

In addition to SLCTs, *DICER1* mutations have also been identified in SCT and other ovarian tumours such as JCGTs, unclassified SCSTs, gynandroblastoma, and rarely in yolk sac tumours, mixed germ cell tumours, dysgerminoma/gonadoblastoma²⁹ and in cervical rhabdomyosarcoma¹²⁴ as well as testicular germ cell tumours.^{103,104,137} No testicular Sertoli

Fig. 5 Sertoli–Leydig cell tumour. (A) Tumour of intermediate differentiation with Sertoli cells arranged in nests, cords and poorly formed tubules and Leydig cells present at the periphery of the Sertoli cells lobules as well as within the oedematous stroma. (B) Retiform variant with papillary and focally retiform growth patterns. (C) Glands lined by gastrointestinal-type epithelium are present within a Sertoli–Leydig cell tumour of intermediate differentiation. (D) Tumour with heterologous (rhabdomyoblastic) elements (top left).

Fig. 6 Sertoli–Leydig cell tumour with the c.5113G>A *DICER1* mutation, encoding an E1705K substitution (arrow). (Figure courtesy of Dr Britta Weigelt.)

cell tumours tested to date have been found to harbour this mutation.¹²⁴ Table 3 summarises the results from various studies evaluating *DICER1* mutation status in different ovarian and testicular tumour types.

No *DICER1* mutations have been identified in any AGCTs tested to date and no ovarian SCSTs have been found to harbour both *FOXL2* and *DICER1* mutations.¹⁰³ Conlon *et al.* detected *FOXL2* mutations in both the GCT-like and SLCT-like areas of the two *DICER1* wild-type gynandroblastomas and the authors noted that there were no apparent morphological differences between the *DICER1*-mutant and wild-type gynandroblastomas, or between *DICER1*-mutant and *FOXL2*-mutant gynandroblastomas.¹²⁴ These findings further support the observation that *DICER1* and *FOXL2* mutations appear to be mutually exclusive in ovarian SCSTs.

Germline *DICER1* mutations have also been reported. This was initially discovered in families with a predisposition for developing pleuropulmonary blastoma as well as other lesions such as cystic nephroma, multinodular goitre and ovarian SLCTs.¹³⁸ It has since been shown that the *DICER1* syndrome also confers an increased risk for other unusual tumours such as cervical embryonal rhabdomyosarcoma, ciliary-body medulloepithelioma, pituitary blastoma, cervical primitive neuroectodermal tumour, juvenile intestinal hamartomatous polyps and Wilms tumour.^{139–142} In contrast to somatic *DICER1* mutations, germline defects are predominantly truncating mutations and are not restricted to ‘hotspot’ regions of the gene. Importantly, it has been shown that SLCTs with germline *DICER1* mutations can also harbour somatic *DICER1* defects,¹⁰³ therefore it has been suggested that referral for genetic testing should be considered in all patients with SLCTs.

SLCTs arising in patients with germline *DICER1* mutation tend to occur at a younger age (first and second decade) than those with sporadic tumours (mean 25 years).¹⁴³ These tumours may exhibit heterogenous morphology with admixed juvenile granulosa cell tumour component or heterologous elements.^{127,143} A review of the SCSTs arising in three patients with germline *DICER1* mutation showed tumours with mixed cytology including sertoliform, JGCT-like,

Table 3 *DICER1* mutation in ovarian and non-ovarian tumours

Reference	Tumours with <i>DICER1</i> mutation
Heravi-Moussavi <i>et al.</i> , 2012 ¹⁰³	26/43 (60%) SLCTs 1/14 (7%) JGCTs 0/15 (0%) AGCTs 2/15 (13%) Yolk sac tumours 0/1 (0%) Dysgerminoma 0/1 (0%) Mixed germ cell tumour 0/5 (0%) Immature teratomas 1/8 (12%) Mature teratomas
De Boer <i>et al.</i> , 2012 ¹³⁷	1/96 (1%) Testicular germ cell tumours
Witkowski <i>et al.</i> , 2013 ¹⁰⁴	8/15 (53%) SLCTs 0/4 (0%) JGCTs 1/7 (14%) Unclassified SCSTs 0/1 (0%) Leydig cell tumour 0/1 (0%) SCTAT 0/20 (0%) Ovarian mature/immature teratomas 0/20 (0%) Ovarian yolk sac tumours 0/1 (0%) Ovarian embryonal carcinoma 2/14 (17%) Ovarian mixed malignant germ cell tumours 1/5 (20%) Dysgerminoma/gonadoblastomas 0/39 (0%) Dysgerminomas 0/1 (0%) Embryonal rhabdomyosarcoma 0/7 (0%) SCCOHTs 0/3 (0%) Testicular mature/immature teratomas 1/7 (14%) Testicular yolk sac tumours 0/1 (0%) Testicular embryonal carcinoma 1/5 (20%) Testicular mixed malignant germ cell tumours 0/3 (0%) Seminomas 20/32 (63%) SLCTs 2/5 (40%) Gynandroblastomas 5/8 (63%) Sertoli cell tumours 0/1 (0%) SCTAT 0/1 (0%) Unclassified SCST 1/4 (25%) Embryonal rhabdomyosarcomas 1/4 (25%) Carcinosarcomas with rhabdomyosarcomatous differentiation
Conlon <i>et al.</i> , 2015 ¹²⁴	0/15 (0%) Testicular SCSTs 6/19 (32%) SLCTs 2/8 (25%) JGCTs 1/12 (8%) Undifferentiated SCSTs 0/101 (0%) AGCTs 0/10 (0%) Fibrothecomas 0/2 (0%) Sertoli cell tumours 0/2 (0%) Steroid cell tumours 0/1 (0%) SST 0/1 (0%) SCTAT 6/10 (60%) SLCTs
Goulvent <i>et al.</i> , 2016 ²⁹	
Kato <i>et al.</i> , 2017 ¹²⁵	

AGCT, adult granulosa cell tumour; JGCT, juvenile granulosa cell tumour; SCCOHT, small cell carcinoma of hypercalcemic type; SCST, sex cord-stromal tumour; SCTAT, sex cord tumour with annular tubules; SST, sclerosing stromal tumour.

and unclassifiable elements, suggesting that the morphological spectrum of SCSTs arising in this setting can be diverse and may not just be restricted to SLCTs.¹²⁷

Management and outcome

The majority of SLCTs are benign with malignant behaviour observed in <15% of patients. Important prognostic factors include stage, degree of differentiation,^{113,144} tumour

rupture^{113,114} and the presence of retiform component¹¹⁷ or heterologous elements.^{111,113,144} Poorly differentiated tumours as well as those with retiform or heterologous elements are generally associated with more aggressive behaviour.¹¹⁴

Most (~80%) patients present with stage Ia disease and can be managed by fertility-sparing surgery, following complete surgical staging, without the need for further adjuvant therapy.^{114,144} More aggressive surgery and chemotherapy are indicated in advanced stage tumours. Adjuvant therapy should also be considered in stage I tumours that are poorly differentiated or contain mesenchymal heterologous elements and for neoplasms of intermediate differentiation that have ruptured.^{145,146} In contrast to AGCTs which are characteristically associated with late disease recurrence, SLCTs generally recur earlier and recurrences are often confined to the pelvis and abdomen.¹¹³

CONCLUSIONS

SCSTs often present as a diagnostic challenge as they encompass a heterogeneous group of tumours with varied and occasionally overlapping morphology. Most pathologists have limited experience with these neoplasms as many of them are uncommon in the routine diagnostic setting. The use of a panel of immunohistochemical markers which are sensitive and specific for sex-cord stromal differentiation, including FOXL2, can help to distinguish these tumours from some morphological mimics, but this is of limited value in differentiating between different subtypes of SCSTs. In instances where a confident diagnosis of an AGCT cannot be rendered based on morphological or immunohistochemical features, FOXL2 mutation analysis should be considered, given the specificity of this mutation for AGCTs. A correct diagnosis of AGCT is important as patients with these tumours have a propensity for late disease recurrences and metastases and require long term follow-up. Similarly, DICER1 mutation is also relatively specific for SLCTs although it is present in a smaller proportion of tumours. The identification of a SLCT, particularly in a young patient, should prompt the pathologist to consider the possibility of an underlying DICER1 syndrome and refer the patient for further genetic work-up. A better understanding of the molecular pathogenesis underlying SCSTs can help improve tumour classification and disease prognostication and also potentially lead to the discovery of more effective treatment strategies which are currently lacking in this group of tumours.

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