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Genetics and Mutational Landscape of Ovarian Sex Cord-Stromal Tumors

Trang Nguyen, Trang Minh Tran, Yee Shen Choo, Maria Alexiadis, Peter J. Fuller and Simon Chu

Abstract

Ovarian sex cord-stromal tumors (SCST) are uncommon tumors accounting for approximately 8% of all ovarian malignancies. By far, the most common are granulosa cell tumors (GCT) which represent approximately 90% of SCST. SCST are also found in the hereditary syndromes: Peutz-Jeghers syndrome, Ollier disease and Maffucci syndrome, and DICER1 syndrome. Key genomic and genetic events contributing to their pathogenesis have been the focus of recent studies. Most of the genomic studies have been limited to GCT which have identified a number of recurring chromosomal abnormalities (monosomy and trisomy), although their contribution to pathogenesis remains unclear. Recurrent DICER1 mutations are reported in non-hereditary cases of Sertoli cell and Sertoli-Leydig cell tumors (SLCT), while recurrent somatic mutations in both the juvenile (jGCT) and adult forms of GCT (aGCT) have also been reported. Approximately 30% of jGCT contain a somatic mutation in the *gsp* oncogene, while a further 60% have activating mutations or duplications in the *AKT* gene. For aGCT, a well characterized mutation in the FOXL2 transcription factor (FOXL2 C134W) is found in the majority of tumors (primary and recurrent), arguably defining the disease. A further mutation in the human telomerase promoter appears to be an important driver for recurrent disease in aGCT. However, despite several studies involving next generation sequencing, the molecular events that determine the stage, behavior and prognosis of aGCT still remain to be determined. Further, there is a need for these studies to be expanded to other SCST in order to identify potential targets for personalized medicine.

Keywords: ovarian cancer, ovary, sex cord stromal tumor, Granulosa cell tumor, FOXL2 C134W, TERT, Sertoli-Leydig cell tumor, DICER1 mutation, transcriptomics, Whole Exome Sequencing

1. Introduction

Ovarian sex cord-stromal tumors (SCST) are a clinically significant group of uncommon neoplasms that represent approximately 8% of ovarian cancers. They are thought to arise primarily from the gonadal sex-cord (granulosa and Sertoli cells) and/or gonadal stromal cells (theca cells) [1]. Malignant ovarian tumors are a group of morphologically, genetically and functionally distinct diseases, but associated with the same organ, the ovary. Epithelial ovarian cancers (EOC)

A. Granulosa-stromal cell tumors
1. Granulosa cell tumor
a. Adult granulosa cell tumor
b. Juvenile granulosa cell tumor
2. Tumors in the thecoma-fibroma group
a. Thecoma
i. typical
ii. luteinized
b. Fibroma
c. Unclassified
B. Sertoli-Leydig cell tumors
1. Well-differentiated
a. Sertoli cell tumor
b. Sertoli cell tumor with lipid storage
c. Sertoli-Leydig cell tumor (tubular adenoma with Leydig cells)
2. Moderately differentiated
3. Poorly differentiated (sarcomatoid)
4. Retiform with heterologous elements
C. Gynandroblastoma
D. Unclassified

^aAdapted from Scully [1] and the 2014 WHO classification [3].

Table 1.
Histological classification of ovarian sex cord-stromal tumors^a.

represent the majority of ovarian cancers (accounting for 85–90%), the other two primary classifications are the SCST and the rarer germ cell tumors [2]. Ovarian SCST are primarily classified histologically as granulosa cell tumors (GCT), Sertoli stromal tumors and SCST of mixed or unclassified cell type, theca-fibroma. In the most recent World Health Organization (WHO) classification of female reproductive tract tumors, SCSTs are separated into pure stromal, pure sex cord and mixed SCST [3] with the sub-classifications of these groups as shown in **Table 1**. GCT are the most common accounting for approximately 90% of all malignant SCST. The clinical and molecular features of GCT has been extensively reviewed by Jamieson and Fuller [2]. Although recurrent and advanced stage GCT are associated with a very high mortality [2], they remain a relatively neglected subset of tumors. The high mortality rate of advanced disease has not been helped by the tendency to group these ovarian cancers with EOC, and apply treatment regimens that are based on therapeutic approaches for EOC, rather than tailoring treatment to the specific SCST [2]. Thus, understanding the genetics and hence the biology of these distinct tumors has an immediacy beyond just understanding tumor biology, with targeted therapeutics urgently needed for women with SCST. In this review we will provide an overview of studies that explore insights into the genetics and genomics of these tumors, with the aim to seek to identify key unanswered questions.

2. Ovarian SCST: clinical, histology and functional aspects

2.1 Granulosa cell tumors

Granulosa cell tumors (GCT) of the ovary are the most common type of SCST, accounting for approximately 5% of all ovarian cancers [4]. GCT are subdivided into two types: the more common adult (aGCT) and the rarer juvenile (jGCT) form. The jGCT subtype represents approximately 5% of all GCT. The two subtypes have different etiologies, and classification for either are not based on age alone as either tumor type can occur at any age. GCT arise from the granulosa cells (GC) of the ovarian follicle, and exhibit many features of normal GC, including expression

of the follicle stimulating hormone (FSH) receptor gene, estrogen synthesis, ER β expression, inhibin subunit expression with synthesis of biologically active inhibin, and anti-Müllerian hormone (AMH) expression [2]. Their presentation may include endocrine manifestations such as features of estrogen excess in prepubertal girls and postmenopausal women. The gonadal peptides inhibin and anti-Müllerian hormone (AMH) can be used in diagnosis and more specifically as tumor markers [2]. Studies from our laboratory as well as those of others have examined gene expression and signaling pathways involved in GC development, and have provided compelling support that not only are GC the cell type of origin for GCT, but that GCT also have consistent features with proliferating GC of the early antral follicle [5].

GCT are classified as low-grade malignancies, that are commonly detected at an early stage, providing a relatively favorable prognosis due to their overt clinical symptoms and indolent course. However, GCT have an unusual propensity for fatal late relapse, ~80% of women with aggressive or recurrent tumors will succumb to the disease [6]. At present, there are no standard methods for predicting relapse, no efficacious targeted therapies (aside from surgery) and no comprehensive understanding of the exact etiology of this disease.

2.2 Fibromas

Ovarian fibromas are the most common benign solid ovarian tumors, they represent 4% of all ovarian tumors. They are well-circumscribed masses that encompass spindle-shaped fibroblastic cells and abundant collagen bundles [1]. Ovarian fibromas can occur at any age but usually after menopause and rarely before 30 years old. The most common recommended treatment is surgery [7, 8]. However, preoperative diagnosis is often difficult due to their solid nature and the lack of specific clinical signs which can result in misdiagnosis as uterine myoma [8, 9]. Ovarian fibromas can also be associated with hydrothorax and ascites causing Meigs' syndrome, a rare condition which is usually misdiagnosed as a malignant myoma [9, 10].

2.3 Thecomas

Ovarian thecoma was first described by Loeffler and Priesel in 1932 who observed that these tumors resembled thecal cells, lutein cells and fibroblasts [11]. Thecoma accounts for 0.5% - 1% of all ovarian cancers. It occurs in mostly postmenopausal women with a mean age of 59 years with only 10% of patients younger than 30 years [12]. Thecomas can be divided into two main types; typical or luteinized, which are thecomas that contain steroid-type cells resembling luteinized theca and stromal cells [12]. The most common symptom experienced by patients is postmenopausal bleeding [13]. The tumors range in size from small to solid masses larger than 15cm [12]. Burnandt *et al.*, found that thecoma tumors were all unilateral; the tumors are well circumscribed and rarely encapsulated, and are often described as yellow-tan, yellow-white or grayish white with no evidence of hemorrhage or necrosis [13].

2.4 Sertoli-Leydig cell tumors

Sertoli-Leydig cell tumors (SLCT) also called androblastomas and arrhenoblastomas, exhibit cellular and molecular markers consistent with a dysgenesis of the ovarian stromal cells, reminiscent of disorders of gonadal dysgenesis [14]. They are rare, accounting for less than 0.5% of all ovarian cancers [3] and can occur in women of all age groups, but they are more often encountered in women under 40 years of age [15]. Patients usually present with symptoms related to androgen excess but can also present with estrogenic manifestations or have an asymptomatic clinical profile. SLCT are typically unilateral tumors and over 97% are diagnosed at Stage 1 [3, 15]. The

prognosis is correlated with the degree of differentiation and stage of the tumor with the five year survival rate of well differentiated SLCT being ~100% [3]. In contrast to GCT, patients with SLCT relapse early, approximately two to three years following initial diagnosis [16]. Many SLCT are associated with somatic or germline mutations in a gene encoding an RNase III endoribonuclease, DICER1, which is involved in the generation of microRNAs (miRNAs) that modulate gene expression at the post-transcriptional level [17–20]. Some studies have reported that 60% of SLCT harbor a DICER1 mutation [21], whereas others have reported that up to 97% of SLCT are DICER1 related [22]. It has been suggested that up to 100% of moderately and poorly differentiated SLCT have DICER1 mutations [17]. A whole exome sequencing study of 17 Chinese patients found somatic mutations in CDC27 (52.6%), DICER1 (21.1%) and MUC22 (21.1%) [23]. Germline and somatic mutations of DICER1 were higher in patients who were younger than 18 years than those in older patients [23].

Taking into consideration that the majority of patients presenting with SLCT are premenopausal with well differentiated tumors at an early stage, fertility sparing surgery with the removal of the affected ovary is recommended [21]. More aggressive surgery and chemotherapy is considered in patients with advanced stage or stage 1 patients with the presence of risk factors such as intermediate and poorly differentiated tumors, heterologous elements, increased mitotic rate, rupture or spillage of the tumor or presence of metastatic tumor [16].

2.5 Gynandroblastomas

The term gynandroblastoma was coined in 1930 by Robert Meyer, who deemed them as an extremely rare variant of SCST comprising of both ovarian (granulosa cell) and testicular (Sertoli cell) histological features [24]. These low-grade hormonally active tumors may also exhibit morphological evidence of stromal theca cells and luteinized cells resembling Leydig cells [24]. Since their first description, only a further 29 cases have been documented [25]. Based on the exceedingly low prevalence of gynandroblastomas, it appears they have a relatively benign disease course [26].

Currently, molecular insights into the histogenesis and pathogenesis of gynandroblastomas are lacking, but it has been postulated that they originate from a single progenitor cell that undergoes differentiation into both female and male elements [27]. This tumor type also shares many clinicopathologic features with other SCST including GCT and SLCT, as previously reported by Jang et al. [26]. Patients typically present with hormonal dysfunction with either estrogenic or androgenic symptoms [28].

The diagnostic criteria for this tumor type stipulate that either Sertoli-Leydig or granulosa cells should comprise at least 10% of the entire tumor mass [29]. There are several sex cord-stromal cell related immunohistochemical markers that exist to facilitate differential diagnoses including inhibin, calretinin, SF1 and CD56, however these are not specific to gynandroblastomas [29]. Other useful diagnostic markers include MART-1/melan-A [30] (specific to Sertoli-Leydig cell and steroid cell tumors), and the cell regulatory protein 14–3-3 sigma [28] (specific to GCT and steroid cell tumors). Further characterization of the molecular pathways mediating the development of gynandroblastomas as well as comprehensive histologic and genetic studies are required.

3. Hereditary syndromes associated with ovarian SCST

3.1 Peutz-Jeghers syndrome

Peutz-Jeghers syndrome (PJS) is associated with ovarian SCST that have histological appearance that is intermediate between GCT and SLCT [31]. The majority

of cases are caused by autosomal dominant germ line mutations in the *STK11/LKB1* (serine/threonine kinase 11/liver kinase B1) gene on chromosome 19p13.3 [32, 33]. It carries a lifetime risk of 21% [32].

LKB1 activates AMP kinase (and its 13 superfamily members), regulating multiple biological processes such as cell polarity, cell cycle arrest, embryo development, apoptosis, and bioenergetics metabolism. LKB1 has become recognized as a critical tumor-suppressor gene that is frequently mutated in a broad spectrum of human cancers. As a tumor suppressor, a number of studies have shown the contributions of the genetic loss of LKB1 to tumorigenesis. The role of LKB1 in controlling cell metabolism through AMPK signaling has been widely documented. The LKB1-AMPK axis controls lipid and glucose metabolism, and acts as a negative regulator of the Warburg effect with the consequence of suppressing tumor growth [34]. Patients with PJS present with gastrointestinal hamartomata, polyposis and both benign and malignant tumors of various organs together with pigmentation of the lips, buccal mucosa and digits [35]. Neither loss of heterozygosity (LOH) at chromosome 19p13.3 nor mutations in the *LKB1* gene have been observed in sporadic ovarian SCST [36, 37].

3.2 Ollier disease and Maffucci syndrome

Ollier disease (OD) and Maffucci syndrome (MS) are both subtypes of enchondromatosis and are considered rare nonhereditary skeletal disorders [38–44], with an estimated prevalence of 1 in 100,000 individuals [45]. They are characterized by multiple enchondromas (benign cartilaginous tumors) and when accompanied with additional subcutaneous soft tissue hemangioma, the condition is referred to as MS [45, 46]. Both disorders can lead to swollen extremities, joint deformities, limitations in joint mobility, scoliosis, and other bone anomalies [47].

OD and MS have been linked to ovarian jGCT, the first reported case of this association dates to 1972 [48], and since that time, a further 16 additional cases have been documented [49, 50]. In 2011 Amary *et al.* demonstrated that >90% tumor patient samples with OD/MS harbored somatic missense mutations in the isocitrate dehydrogenase (IDH) 1 and 2 genes, 65% of which encodes a R132C amino acid substitution on exon 4 [51, 52]. The mutant IDH gene produces the potential ‘onco-metabolite’ 2-hydroxyglutarate (2-HG) which induces histone hypermethylation [45, 51, 53]. The role of either the mutant IDH variant or 2-HG in the pathogenesis of OD/MS needs to be further explored, however they may represent an early post-zygotic event which has implications in tumorigenesis [51, 54].

4. Genomic changes in ovarian SCST

As previously mentioned, studies of changes at a genomic level in ovarian SCST have largely been restricted to aGCT. In contrast to EOC, GCT have a relatively stable karyotype [55]. Cytogenetic analysis [56] and comparative genomic hybridization (CGH) [57] studies have revealed trisomy of chromosomes 12 and 14 in approximately one third of aGCT cases and a similar percentage of monosomy of chromosome 22 [56, 57]. Between 5% and 20% of aGCT are aneuploid, however, neither the karyotype nor ploidy provides prognostic information [56, 58–60]. Mutations of lesser frequency have been observed at other loci, again providing no prognostic significance.

In a study by Caburet *et al.*, who applied CGH to a panel of aGCT, as well as collating data from a total of 94 aGCT from previous studies [61], they observed that a total of 64 tumors had large-scale chromosomal changes. Supernumerary

chromosomes 8, 9, 12 and 14 were reported, with the latter being very common (25 of 64). Partial or complete loss of chromosomes 1p, 13p, 16, 11 and 22, with monosomy 22 were also very common (36 of 64). There was co-occurrence of chromosomal alterations although there was only a statistically significant non-random association for +14 with -22 and +7 with -16q. Further, Caburet *et al.* combined transcriptomic data from a previous study [62], seeking to identify gene copy number changes that may reflect putative driver changes in the pathogenesis of aGCT [61]. Twenty genes were identified from the regions of chromosomal imbalance with a plausible, pathological role across nine chromosomes (1, 5, 11, 12, 14–17, 22) including the *AKT1* gene being the most frequently amplified (6 of 10 tumors) and the nuclear receptor, rev-erbA α being the second most frequent (5 of 10 GCT). The latter is consistent with the findings of our previous study examining gene expression of all 48 nuclear receptors in aGCT [63]. Caburet *et al.* also sought to identify recurrent ‘broken’ genes (the presence of a mapping breakpoint within the genes in two or more tumors). They observed that five genes fitted this criterion on 5 different chromosomes. The authors [61] speculated on the potential of these genes in driving the pathogenesis of GCT, while recognizing the limitation of the study where the correlation set comprised of only ten aGCT, nine of which were stage one disease [61].

For other SCSTs, reports of cytogenetic analyses are extremely scarce. A recent clinical case report describes three patients, from two unrelated families, with 14q32 deletions encompassing the *DICER1* locus. Two of these patients have a history of *DICER1*-related tumors, including a 15-year-old female with a SLCT [64]. For thecoma-fibromas, a report by Streblow *et al.* found that trisomy 12 is a non-random chromosomal abnormality, while gain of chromosome 9 and loss of chromosome 4 and/or 9 were features of fibromas [65]. Loss of chromosome 9 copy number in a subset of the fibromas analyzed is noteworthy because of the association of ovarian fibromas and Gorlin-Goltz syndrome or nevoid basal cell carcinoma [66]. Gorlin-Goltz syndrome is an autosomal dominant disorder featuring distinctive congenital malformations and a predisposition to a variety of benign and malignant neoplasms, including ovarian fibroma [67]. The gene for Gorlin-Goltz syndrome, *PTCH1*, has been localized to 9q22.3 and is characterized as a tumor suppressor gene encoding for a transmembrane protein that functions as a receptor for sonic hedgehog [68]. LOH of one chromosome 9 homolog in three non-syndromic ovarian fibromas suggests a somatic role of the *PTCH1* tumor suppressor gene in these neoplasms. Additional studies of sporadic and syndromic ovarian tumors of the thecoma-fibroma group using other approaches may expose an even higher frequency of *PTCH1* loss or mutation.

4.1 Somatic genetics of jGCT

Juvenile GCT (jGCT), as with aGCT, exhibit macroscopically a mixture of solid and cystic components with hemorrhagic areas. Thus, it is difficult to differentiate jGCT and aGCT by radiologic and morphologic findings. However, their histology differs from aGCT with a follicular or diffuse pattern of larger luteinized cells [69]. JGCT follicles have various sizes and shapes containing basophilic secretions. The cells have rich eosinophilic and/or vacuolated cytoplasm (indicating luteinization) and indistinct cell borders. They contain round, hyperchromatic or markedly bizarre nuclei which lack the nuclear grooving characteristic of aGCT [2, 69]. Unlike aGCT, Call-Exner bodies are not a feature of jGCT. The mitotic rate is high with marked nuclear atypia [2, 26]. Although the histologic appearances are therefore more ‘aggressive’ than for aGCT, the prognosis is generally better. The distinction between aGCT *vs* jGCT is therefore primarily based on the histology.

This by itself can create diagnostic dilemmas, however, these are increasingly being resolved by the use of the molecular markers, which are discussed below [70–72].

The gene expression profile of GCT are similar to an FSH-primed proliferating preovulatory GC [5]. FSH stimulation of GC growth is mediated by the FSH receptor, a G-protein-coupled, seven-transmembrane domain receptor. We and others have hypothesized that activation of these pathways, perhaps through activating mutations in these signaling molecules of the FSH signaling pathway, may play a role in the pathogenesis of GCT as is common in other endocrine tumors [2]. Despite extensive investigations, this does not appear to be the case for aGCT. However, mutations were found in the *gsp* oncogene in approximately 30% of jGCT [73]. The activating mutations at position 201 of the stimulatory alpha-subunit of the heterotrimeric G-protein ($G\alpha_s$), which couples with seven-transmembrane domain receptors such as the FSH receptor, have been reported as somatic mutations in pituitary, thyroid and adrenal tumors as well as being the inherited mutation in the McCune–Albright syndrome [74]. In jGCT, the mutation is either R201C or R201H, and reported to be associated with a poorer prognosis [73].

In addition, it has been postulated that as the FSH receptor signals through the oncoprotein AKT, that mutations in this signaling pathway may contribute to the pathogenesis of jGCT [75]. Indeed, in one study, >60% of jGCT had an in-frame duplication of the plekstrin-homology domain leading to activation of AKT1. Other AKT1 point mutations of uncertain significance were also observed in jGCT. It was speculated that the resulting mutated AKT1 proteins are hyperactive with increased membrane association of AKT1, resulting in constitutive FOXO3 repression [75]. A subsequent study using transcriptomic analyses found that the changes in gene expression in these tumors may reflect a limited set of transcription factors altered by AKT1 activation [76].

4.2 Somatic genetics of aGCT

Many cancers develop from somatic mutations in driver genes that occur sporadically during replication or as a result of environmental factors and are not inherited. It is therefore important for the development of new therapeutic techniques to identify and consider how somatic mutations accumulate in cancer genomes. In 2009, Shah *et al.* described a somatic missense mutation in the *FOXL2* gene that was found in >97% of aGCT examined [55]. Their approach utilized whole transcriptome paired-end RNA-sequencing (RNA-Seq) to analyze four aGCT. They identified a somatic missense mutation in codon 134 (402C → G) that results in the substitution of a highly conserved cysteine residue by tryptophan. Numerous studies, including our own (reviewed in Ref. [2]), have confirmed this finding [55]. Both heterozygosity and hemi-homozygosity of this mutation are also reported [2, 55]. The mutation is unique to aGCT and has not been observed in jGCT [2]. The rare exceptions to this rule appear either to be mixed tumors in which elements are in fact of GC origin or the occasional tumor which truly is ‘the exception to the rule’ [70].

The presence of the *FOXL2* C134W mutation provides a clear distinction between jGCT and aGCT. In jGCT, *FOXL2* expression is low or absent [70, 77], whereas in aGCT expression levels in tumors bearing the mutation are generally consistent with levels seen in the normal ovary [70]. *FOXL2* expression in heterozygous tumors appears equivalent for the wild-type and mutant *FOXL2* alleles. In jGCT, low or absent expression of *FOXL2* is associated with aggressive disease and carries a poor prognosis. The presence of the *FOXL2* C134W mutation provides a molecular diagnosis of aGCT which has proven helpful in resolving the diagnosis of aGCT in histologically ambiguous or problematic cases [70–72].

FOXL2 plays a fundamental and essential role in ovarian development; its biology has been extensively studied [78–80]. It is a member of the forkhead box (FOX) family of evolutionarily conserved transcription factors. The C134W mutation is predicted to lie close to, but not in the DNA-binding domain [55]. Despite an extensive understanding of the biology of FOXL2 [78–80], the mechanisms of the tumorigenesis mediated by this somatic mutation in aGCT remain to be clearly established. *In vitro* evidence indicates that it impacts both steroidogenesis and apoptosis in GC [79]. In addition, post-translational modifications (sumoylation, phosphorylation, acetylation and ubiquitinylation) may also play a critical role in the modulation of FOXL2 function [78, 79]. Kim *et al.* (45) reported increased phosphorylation of FOXL2 as a result of the C134W mutation, subsequently leading its ubiquitinylation and degradation. The mutation would likely impact on critical protein–protein interactions of FOXL2, but these remain to be clearly elucidated. Caburet *et al.* argues that FOXL2 is a tumor suppressor gene with loss-of-function being associated with malignancy, as is seen in jGCT, and therefore the C134W mutation compromises function rather than being associated with activation or gain of function [78]. Conversely, others have argued that FOXL2 may act as a tumor suppressor gene in jGCT but the FOXL2 C134W mutation may be oncogenic in aGCT [80]. Its role is likely to be more complex than a simple loss-of-function, as one would speculate that other inactivating mutations in the FOXL2 gene would have been identified in aGCT [2]. It may be reminiscent of the DICER1 mutation in SLCT where one facet of DICER function is selectively lost [81]. It is also curious that aGCT express the wild-type FOXL2 allele at equivalent levels to the mutant allele, a scenario which arguably affirms that the mutant FOXL2 must be ‘dominant negative’ if there is suppression of function.

Although the majority of aGCT are stage 1 tumors and cured by surgical resection, those who have advanced stage disease or recurrent disease carry a poor prognosis [2]. As the FOXL2 C134W mutation is present in the vast majority of all aGCT, it does not explain differences in stage or behavior. It may be, as with certain inherited mutations, e.g., the ret. proto-oncogene in medullary thyroid cancer [82], that the transition from ‘hyperplasia’ induced by the somatic mutation to frank malignancy requires a second independent hit. Evidence to date indicates that this second event may be less specific than the first. In the case of aGCT, the genomic changes described above may for instance reflect the ‘second hit’ that results in aggressive clonal expansion. The subsequent somatic mutations that presumably drive tumorigenesis, recurrence, aggressive behavior, transcoelomic spread and metastatic disease still remain to be fully elucidated.

4.3 The GCT transcriptome

Evidence provided by recent transcriptomic studies have elucidated the genes whose expression has been modified, in some instances, may reflect genomic rearrangements. Gene expression microarray was used by Benayoun *et al.* comparing 10 aGCT with two GC samples acquired during *in vitro* fertilization (IVF) egg retrieval [62]. In principle, IVF provides a ready source of ‘normal’ tissue to be used as a control, however, the limitation of this control is that the GC are collected after IVF cycles involving a hyperstimulation regimen with gonadotropin, and hence the GC being partially luteinized at the time of collection [5]. Thus, these controls do not reflect GCs from the proliferative phase [5]. The authors identified genes involved in cell proliferation and a decrease in expression of genes that promote apoptosis [62]. Interestingly, the group showed modulation of genes that are known to be FOXL2 targets. Genes typically down-regulated by FOXL2 but increased in this context, were those associated with tumorigenicity. Conversely, genes usually

upregulated by FOXL2 and associated with apoptosis were down-regulated. Hence, it was suggested that the FOXL2 C134W mutation causes a partial loss-of-function suggesting it is a tumor suppressor gene. This notion is consistent with jGCT also lacking FOXL2 expression as previously mentioned [78].

Our laboratory has generated transcriptomic profiles between a cohort of six stage 1 and six stage 3 aGCT patients using a gene microarray approach to reveal significant differential gene expression between early and advanced stages. All of the aGCT samples were sequenced and also found to be heterozygous for the FOXL2 C134W mutation [83]. A total of 16 genes were reported as highly abundant in the advanced aGCT, with a further 8 genes found to be more highly expressed in the stage 1 aGCT (p value <0.05, >2fold-change). Curiously, two genes associated with malignancy were found to be highly expressed in the advanced stage aGCT, a member of the cytokine family called CXCL14 (chemokine C-X-C-motif ligand 14), and a multifunctional secretion protein called MFAP5 (microfibrillar-associated protein 5 transcript variant 1), which were 40- and 26-fold higher, respectively. Of the genes whose expression was high in the stage 1 aGCT, INSL3 (insulin-like 3 transcript variant 2) gene expression was 75-fold higher in stage 1 aGCT and provided robust discrimination of the two groups [83]. Whether INSL3 inhibits tumorigenesis or whether the diminished expression in advanced stage disease is simply a marker of de-differentiation of the tumor remains to be determined. Applying Gene Set Enrichment Analysis (GSEA) to these data sets [83] showed increased expression of genes on chromosome 7p15 in the stage 3 aGCT, which is consistent with the report of Lin *et al.* [57] found using CGH, gain of chromosome region 7p15-p21 in some aGCT samples.

4.4 The genomic landscape of GCT

Aside from the identification of the FOXL2 C134W mutation in GCT, there have been several studies that have aimed to identify genomic alterations through sequencing candidate genes and known oncogenes [2]. Genes commonly mutated in other malignancies such as p53, PI3K, RAS and BRAF, are not a feature in GCT, and thus, putative 'second-hit' mutations still remain to be identified. But specific. The approach taken by The Cancer Genome Atlas project (TCGA) where a defined cohort of tumors are subjected to a full suite of genomic analyses [84] has yet to be applied to aGCT or indeed to other ovarian SCST.

The critical challenge to be addressed as a precursor to both improved prognostication (predicting recurrence) and identification of GCT-specific therapeutic targets (to address the high mortality of advanced disease) is to identify the molecular drivers of GCT pathogenesis beyond the aetiologic FOXL2 mutation.

In our own whole exome sequencing (WES) study, DNA from 22 fresh frozen, FOXL2 C134W mutation-positive GCT (14 stage 1 and 8 stage 3) was sequenced [85]. The analysis identified on average 64 coding and essential splice-site variants in each tumor, however recurrent mutations were not identified in individual genes or in related genes. The genes that were identified to contain truncating (stop, gain or frameshift) mutations, essential splice site mutations, non-synonymous mutations and stop/loss mutations in the stage I (970 variants) and recurrent (434 variants) tumors, were subject to variant effect pathway analysis. The canonical pathways identified were linked to DNA replication and/or repair as might be expected in malignancy; and to signaling through the epidermal growth factor receptor (EGFR) family. We also identified a high frequency of a TERT promoter mutation (see below).

Hillman *et al.* [86] reported a comparable outcome for adult GCT subjected to WES [86], in a study that focused on truncating mutations of the histone lysine

methytransferase gene KMT2D (also known as MLL2) as a recurrent somatic event. They reported these mono-allelic KMT2D-truncating variants to be more frequent in recurrent (23%) compared with primary (3%) GCT when an expanded GCT cohort was examined. KMT2D is a tumor suppressor gene that is the target of frequent inactivating mutations in several tumor types, including medulloblastoma and lymphoma. Interestingly, these mutations did not correlate with loss of protein as determined by immunohistochemistry (IHC). We found heterozygous KMT2D frameshift variants in only three (2x stage 3) of 22 GCT in our cohort [85] and Zehir *et al.* (see below) reported two frameshift variants in 11 GCT [87]. Hillman *et al.* [86] did not determine the TERT promoter mutation status of their GCT cohort.

Zehir and colleagues determined the mutational landscape in tumors from 10,000 patients using their targeted MSK-IMPACT panel of 341 cancer associated genes; within this study, there were 11 FOXL2 mutation-positive GCT (two primary and nine “metastasis”) [87]. They identified mutations in 17 (5%) of the 341 cancer-associated genes on the array in these GCT samples; in only four of these genes was the mutation also found in our WES study [85].

In a recent study by Pilsworth *et al.*, the authors used a combination of whole genome sequencing and targeted sequencing [88], and reported a similar frequency of KMT2D inactivating mutations as that of the Hillman *et al.* study [86] (10.8% compared to 13.9%). The difference between the two studies however was that in this study, there was no association of the KMT2D mutation with recurrence [88]. This is consistent with another published study [89] which also showed no association of this gene mutation with recurrent disease. The low frequency of this mutation in these studies as well as our own, suggests that they may be pathogenic driver mutation in only a subset of aGCT. Additional inactivating mutations were also identified in low frequency, including the candidate tumor suppressor gene WNK2 and a newly discovered protein called NLRC5, which has been linked to the regulation of cancer immune evasion [88].

In another study, TP53 mutations were identified in 9.1% of patients, with higher tumor mutational burden and mitotic activity [90]. These findings suggest that tumors harboring TP53 mutations may be a high-grade subgroup of aGCT. It is noteworthy however, that other studies have not observed mutations in TP53 at similar frequencies [2, 88].

Indeed, the lack of overlap in the mutational variants identified in these various studies is curious. Also, somewhat surprising is the very limited number of recurrent mutations in specific genes, given that, by many criteria [83, 91], including the pathognomonic mutation in the FOXL2 gene [70], GCT are remarkably homogenous. It is conceivable that the lack of clear driver mutations may indicate that the key drivers are: 1) as in other cancers, including endocrine cancers, gene fusion events (splice-variants and translocations) which contribute the “second hit”; or that in ~40% of GCT, TERT mutations are an important tumorigenic event with perhaps loss of KMT2D in a small subset.

4.5 TERT promoter mutation

Our WES study [85] confirmed the report, from Pilsworth *et al.*, of a telomerase gene (TERT) promoter mutation [92]. The TERT gene encodes the catalytic subunit of telomerase; TERT transcriptional regulation is the limiting step in telomerase activity. Elongation and/or preservation of telomere length is regarded as a hallmark of cancer. Two hot-spot mutations in the telomerase promoter, -124C > T and -146C > T are commonly found in specific cancers: melanoma, glioblastoma, bladder cancer and thyroid cancer, but not in common epithelial cancers, such as breast and prostate [87]. Our analysis using targeted PCR identified 11 of 26 (i.e., 42%)

of the GCT in our analysis to be heterozygous for the -124C > T TERT promoter mutation - a frequency that matches the above cancers [87]. 29% of the stage 1 GCT were heterozygous for the mutation, while 67% of the stage 3 GCT contained the mutation [85]. The -124C > T mutation is also present in the aGCT-derived KGN cell line [85]. There are *in vitro* data that the two promoter mutations are not equivalent [93], suggesting that in GCT there is a tumorigenic advantage only for the -124C > T promoter mutation.

Increased telomerase activity appears also to be associated with cell proliferation independent of telomere lengthening [94]. TERT has been reported to interact with major oncogenic signaling pathways including c-MYC, NFκB, and Wnt/β-catenin. Of these, activation of NFκB signaling has been reported in the KGN cell line [91, 95] and p65 nuclear localization has been reported in GCT [96], although previous studies [85, 86, 88, 90] have not identified mutations in these pathways.

It has been noted that melanoma, glioma, and papillary thyroid and bladder carcinomas, all of which have a high frequency of TERT promoter mutations, are characterized by activation through BRAF or EGFR mutation of the MAPK signaling pathway [97]. This association is intriguing given this high frequency of the TERT promoter mutation in GCT and the suggestion from pathway analysis of the WES study linking one of the canonical pathways to signaling through the EGFR family [85]. The high incidence of the TERT promoter mutation in GCT, together with the correlation of the presence of this mutation with stage, suggests that the presence of the TERT promoter mutation, as in other tumors, may be of prognostic and/or pathogenic significance, and acquired during tumor progression after the initial FOXL2 driver mutation.

4.6 DICER1 syndrome

DICER1 syndrome is a rare inherited disorder that increases the risk of a variety of cancerous and non-cancerous tumors that occur in the lungs, kidneys, ovaries and thyroid. DICER1 syndrome results from germ-line mutations in the *DICER1* gene, located on chromosome 14, position q32.13, encodes an RNase III endoribonuclease which plays a critical role in processing micro(mi)RNA to their mature forms. DICER1 contains two highly conserved RNase III domains (RNaseIIIa and RNaseIIIb) which forms a catalytic dimer, creating a single processing center for dsRNA cleavage, with each RNase III domain cleaving one strand of the dsRNA resulting in miRNA named by their prime end origin (3p/5p miRNA) [98]. Germ line and somatic mutations in the *DICER1* gene have been described in ovarian SCST, predominantly for SLCT. DICER1 mutations were initially reported to cause familial pleuro-pulmonary blastoma, but have been subsequently found in a variety of tumors, including ovarian SLCT and in association with benign thyroid pathologies [20]. The mutations occur in approximately 60% of ovarian SLCT of which 80% are the p.E1705K mutation [19, 20]. DICER1 mutations are also seen in gynandroblastomas. They have not been associated with GCT or, testicular stromal tumors [19, 20, 72]. The functional consequence of DICER1 mutations is there is a bias caused by the mutated DICER toward processing of the RNaseIIIa strand of the miRNA duplex [19, 81]. Thus, there is a selective reduction in RNaseIIIb activity and retention of RNaseIIIa activity, resulting in an excess of 3p-miRNA and a depletion of 5p-miRNA [19, 81, 98]. One copy of the altered gene is sufficient to cause an increased risk of developing tumors. Although a mutation in the *DICER1* gene can infer an increased chance of developing SLCT, many individuals who carry a mutation in the *DICER1* gene do not necessarily develop tumors [99]. The therapeutic or diagnostic value of these mutations for SLCT warrants further investigations.

5. The 'miRNA-ome' and other non-coding RNAs

A pathogenic role for miRNA in SCST can be indicated by the identification of aberrant miRNA processing in SLCT and gynandroblastomas. However, studies of the 'miRNA-ome' have been limited. Rosario *et al.* profiled miRNA expression and regulation in the KGN and COV434 cell lines [100]. They observed that COV434 cells preferentially expressed miR-17 family members whereas the KGN cells preferentially expressed members of the let-7 miRNA gene family [100]. There has not however, been any systematic studies in GCT or, to our knowledge, for other SCST.

Long non-coding (lnc) RNA's have also been implicated in oncogenesis [101]. Evidence indicates that lncRNA can produce short peptides from small open reading frames (smORFs) which can regulate biological processes [102]. The status of both lncRNA, and indeed, smORFs remains to be investigated in SCST.

6. GCT-derived cell lines

The human KGN and COV434 cell lines, have been thought to be derived from GCT, and are extensively used in studies of GCT as well as to model normal GC function. Both cell lines exhibit some features that are reminiscent of normal proliferating GC, including a functional FSH receptor and aromatase activity. Jamieson *et al.* analyzed the FOXL2 status of both cell lines [70], concluding the COV434 cells lack FOXL2 expression and indeed the C134W mutation, lending to the assumption that they are derived from a jGCT [70]. In contrast, the KGN cell line (established from a metastatic aGCT), expresses FOXL2 and is heterozygous for the FOXL2 mutation, which is consistent with it being derived from an aGCT [70]. Both cell lines were established from patients with advanced aggressive disease.

Both KGN and COV434 cell lines are notable for constitutive activity of the NF κ B and Braf/ERK signaling pathways [91, 95, 103]. A molecular study using a transcriptomic approach conducted by Rosario *et al.* was used to identify potential targets of FOXL2 in KGN and COV434 cells [104]. They observed that many of the genes regulated by wild-type FOXL2 were also regulated by the mutant FOXL2, notably genes involved in the transforming growth factor-beta (TGF- β) signaling pathway. Their analysis also highlighted the significant differences between the COV434 and the KGN gene-expression profiles [104]. In our transcriptomic analysis of aGCT [83], we observed over 3000 entities that differed greater than twofold (p value of <0.05) when 12 aGCT were compared with the KGN cells. This was in stark contrast to only 24 differentially expressed genes observed when comparing the stages 1 and 3 aGCT. Thus, although the two cell lines are valuable tools in the analysis of signaling pathways in the context of both GCT and indeed GC, they do not assist in the genomic and/or genetic analysis of aGCT.

The classification of COV434 as a GCT-derived cell line has been questioned. Recent studies show that this cell line was likely derived from a small-cell carcinoma of the ovary hypercalcemic-type (SCCOHT) [105–107]. The cell of origin of these tumors is unknown, with reports postulating they are likely derived from the germ cells [108]. Recent advances in molecular genetics have indicated that SCCOHT can be regarded as an ovarian malignant teratoid/rhabdoid tumor (MRT) [109]. SCCOHT are characterized by the loss of both SMARCA2 and SMARCA4, which are also not expressed in COV434 cells [105, 107]. Moreover, the lack of expression of RUNX2 and high expression of RUNX3 in COV434 suggests that these cells do not represent primary jGCT [106]. Noticeably, the study of Karnezis *et al.* indicates that COV434 cell line has all morphological, immunohistochemical, genetic and clinical

characteristics of SCCOHT [107]. They also noted that the level of serum calcium in mice increases when transplanting with COV434 [107].

7. Animal models of ovarian SCST

A number of mouse models in which GCT arise have been reported, however none truly recapitulate the human disease [2, 110]. Liu *et al.* have described the development of GCT in mice with conditional inactivation of FOXO1/3 in GC [110]. The development of these tumors was accelerated with perturbation of the multi-functional tumor suppressor gene PTEN. An examination of PTEN and FOXO1/3 expression in five primary human aGCT samples found low expression for each [110], leading them to conclude that this mouse model, in contrast to others, shares some characteristics with aGCT. Arguably however, involvement of PTEN in the model, is more consistent with activation of PI3K/AKT which is more of a feature of jGCT. It should be noted that neither mutation, over-expression of PIK3CA or PIK3R1, nor loss of expression of PTEN, has been reported in aGCT [111]. Work from Lague *et al.* has provided evidence in mouse models for a synergistic effect of the Wnt/ β -catenin and PI3K/AKT pathways in the formation of GCT, which is of interest given the potential role for AKT1 mutations in jGCT [112]. Wnt/ β -catenin signaling pathway has well established roles in ovarian development and in GC function [2]. Although dysregulation of Wnt/ β -catenin signaling has been identified in many human cancers, there is no evidence for activation of this pathway in human GCT [113, 114], which contrasts to equine GCT where there is clear evidence of Wnt/ β -catenin signaling activation [113]. Increased ovarian R-spondin1 signaling, which modulates Wnt signaling is associated with GC-like tumors [115]. Gao *et al.* targeted expression of a constitutively activated TGF- β receptor to GC and found GCT that were associated with elevated inhibin and estrogen levels [116] as is seen in human GCT which perhaps more closely recapitulates the clinical situation than earlier models in which inhibin gene deletion resulted in GCT (see below) [117]. One of the downstream consequences of this activation is again, increased AKT signaling. The knockout of the inhibin α subunit (shared by both inhibin A and B) causes the development of SCST in mice of both sexes as early as four weeks [117–119]. In these inhibin α null mice, FSH levels has increased by two to three fold which correspond to inhibin's physiological function to suppress FSH [118]. However, a double knockout of inhibin α and FSH unexpectedly showed development of gonadal tumors in the mice; the tumors developed after 12 weeks of age [117–121]. The inhibin α knockout led to increasing levels of activin which induce the activation of SMAD2/3 signaling pathway in GC [117, 121]. The study of Madh3 (SMAD3-null) and inhibin α double knockout mice demonstrated slow progression of tumor growth; SMAD 3 is thus important for tumor progression [121–123].

8. Treatment strategies for SCST

The uncommon nature of SCST limits the ability to develop targeted therapies and evaluate them in well-powered clinical trials. A recent search of clinicaltrials.gov showed only 11 trials that are either active or recruiting involving SCSTs, with only five completed results described. The application of new sequencing technologies may lead to the discovery of novel driver genes that lead to these rare ovarian cancers. However, as discussed above, these have so far been elusive from the limited studies performed to date.

8.1 Treatment of GCT

Surgical treatment is the mainstay for peri- and postmenopausal women diagnosed with aGCT, with total abdominal hysterectomy (TAH), bilateral salpingo-oophorectomy (BSO) and full staging surgery thought to be the most appropriate initial treatment [124]. Randomized trials of adjuvant chemotherapy are not available, and for patients with poor prognosis, adjuvant platinum-based chemotherapy is generally considered either alone or in combination with doxorubicin and cyclophosphamide (CAP) [125, 126], vinblastine and bleomycin (PVB) [127], etoposide or etoposide and bleomycin (BEP) [128, 129]. The use of these treatment regimens is often based on those employed for epithelial ovarian cancer and in the main have proven to be of limited benefit [130].

Hormone treatment has shown promise in the treatment of advanced GCT based on their frequent estrogen dependence [2, 131, 132]. A systematic review of hormonal therapy for GCT revealed a pooled response rate of 71% and aromatase inhibitors (AI) were identified by far the most effective agents [131]. In a more recent study, the use of AI in 25 cases with known outcomes, the response rate to AIs was 48% (12/25) and the clinical benefit rate was 76% (19/25) [132]. Although these numbers are limited, they indicate the use of AIs as a potential alternative to chemotherapy, although the mechanisms involved in GCT sensitivity to AIs remains undefined. Other forms of hormone therapy have also previously shown promise with reports of prolonged remission (14–42 months) documented in patients with extensive disease treated with high doses of medroxyprogesteroneacetate [133, 134].

The expression of vascular endothelial growth factor (VEGF) appears persistent with most GCT, with almost all tumors (93%) showing positive VEGF immunostaining in one study [135, 136]. The use of the anti-VEGF-A monoclonal antibody, bevacizumab, was shown to cause apoptosis in GCT-derived cells *in vitro* [136]. Extending this to a small retrospective study showed promising activity with bevacizumab in 8 women with recurrent GCT [137]. There was one complete response in an overall response rate of 38%, with the clinical benefit rate being 63%. Bevacizumab is also effective in treating ascites in recurrent GCT, reflecting the role of tumor-derived VEGF in the formation of cancer-related ascites [138]. This led to a prospective phase II clinical trial of bevacizumab in relapsed aGCT which reported a 16.7% response rate and median progression free survival of 9.3 months (95% CI 4.1–15 months) in the 36 patients recruited [139].

Tyrosine kinases are well recognized as being fundamental to many growth factor signaling pathways in both normal and malignant cells. The advent of specific inhibitors of tyrosine kinases (TKI) has focused attention on the potential of TK as therapeutic targets. In view of the evidence of activation of cell signaling in GCT and a case report of a recurrent GCT responding to the TKI, imatinib (Gleevec), our group demonstrated that the GCT-derived cell lines were inhibited by imatinib and indeed by the newer more potent analog, nilotinib, but at concentrations higher than those required for the targeted receptor kinases [140]. The AP-1 signaling pathway is also constitutively activated in GCT [95]. We tested a TKI, sorafenib (Nexavar, Bayer), which has high affinity for Raf-1 and Braf, in addition to the above-mentioned TK, and found that this TKI elicits a dose dependent inhibition of both cellular proliferation and viability in both cell lines at concentrations equivalent to that seen in other systems [141]. A commercially available Raf-1 kinase inhibitor was also examined and found to have no effect on cell proliferation and viability in both cell lines, thus implicating Braf in the activated AP-1 signaling [141]. Based on these data, clinical investigation of sorafenib or possibly a more potent BRAF inhibitor, such as vemurafenib or dabrafenib, may be warranted.

Little is known about the immune response in SCST. Expression of the immune checkpoint protein, programmed death-ligand 1 (PD-L1) has been reported only in abstract form, and present in ~75% of SCSTs [142], however, immunotherapy has not been reported in a clinical trial for these tumors. A more recent study by Pierini et al., suggests that tumor infiltrating lymphocytes (TILs) are the main immune population in GCT [143], and that after *ex vivo* expansion of TILs isolated from 11 GCT patients, showed they vigorously reacted against autologous tumors (100% patients) and against FOXL2 peptides (57.1% of patients). This suggests that FOXL2 immune targeting can produce substantial long-term clinical benefits and lay a foundation for future trials testing immunotherapeutic approaches toward GCT [143].

Based on several studies, there is also the potential for more targeted therapies that arise from identifying the molecular mechanisms that contribute to the pathogenesis of GCT. The NF κ B signaling pathway is often involved in cancer development; activated NF κ B increases the expression of genes involved in cell proliferation, metastasis, angiogenesis and anti-apoptosis [144]. Apoptosis is directed by activated caspases. The Inhibitors of Apoptosis (IAP) proteins suppress apoptosis through the inhibition of the caspases. The cellular IAP1 (cIAP1 or BIRC2), cellular IAP2 (cIAP2 or BIRC3) and X chromosome-linked IAP (XIAP or BIRC4) are the main IAPs with known roles in apoptosis and cancer [145–147]. XIAP is the best characterized and also the most potent caspase inhibitor, blocking both intrinsic and extrinsic apoptotic signals by directly inhibiting caspases-3, -7 and -9. cIAP1 and cIAP2 have less potent roles in opposing these pathways as they do not directly bind caspases, however they can indirectly cause caspase cleavage [145–147]. Inhibition of cIAPs and XIAP causes cells to become more receptive to both intra- and extracellular apoptotic signals [148]. XIAP is predominantly regulated by an endogenous mitochondrial protein called second mitochondria-derived activator of caspases (Smac), which is released during apoptosis, and interacts with XIAP through conserved amino acid residues in the BIR3 domain of XIAP to antagonize XIAP-mediated caspase inhibition [149].

Due to its elevated expression and prominent ability to inhibit cell death, XIAP is an attractive therapeutic target for anti-cancer treatment [145–147]. Smac-mimetics (SM) bind directly to XIAP with high affinity to prevent caspase binding, thus neutralizing XIAPs pro-oncogenic function. A number of Smac-mimetics have demonstrated good anti-cancer activity in preclinical studies, and several have already passed primary phase clinical trials, suggesting that these compounds are well tolerated [146]. Though XIAP, IAP or pan-IAP inhibitors have shown some efficacy as single agents, the majority of studies have shown more promise when used in a rational drug combination strategy [146]. We have shown *in vitro* and using GCT explants in culture, that targeting XIAP as a combination therapy with activation of the peroxisome proliferator-activated receptor-gamma protein (PPAR γ) provides a novel and specific therapeutic strategy for GCT [150, 151]. It remains to be determined the effectiveness of this combination approach in *in vivo* studies.

9. Conclusions

Recent genetic discoveries have provided profound insights into the molecular pathogenesis of ovarian SCST. As with other uncommon tumor types, insight from research of SCST will potentially be prismatic; that is, it will help clarify molecular mechanisms involved in oncogenesis. In SLCT, the discovery of DICER1 mutations highlight both the complexity and asymmetry of miRNA processing, while also

supporting the potential for ‘non-coding’ RNA in playing a critical role in malignant cancers. In the case of jGCT, the presence of the recurring mutations in the *gsp* oncogene and in AKT1, highlights the critical role of the cyclic AMP/protein kinase A and PI3kinase/AKT signaling pathways in hormone-mediated cell proliferation, as well as when constitutively activated, in malignancy. We and others have demonstrated that the FOXL2 C134W mutation found in aGCT would appear to be pathognomonic, however, the precise mechanism of this mutation still remains somewhat controversial, despite being discovered over a decade ago. For other SCST, gene alterations and mutations appear restricted to their syndromic context. The above findings have provided insights into the biology of the respective genes involved in the pathogenesis, and to the role they play in sex-cord stromal cell development. The prognostic significance and therapeutic potential of these findings are of critical interest to those women afflicted with these malignancies. What is also very clear is that these tumors are uniquely different to the EOC, which in the context of the age of ‘precision’ medicine, each tumor type must be treated with a tumor-, and/or a mutation-specific approach. As an example, for the more common aGCT tumor type, advanced stage disease carries a poor prognosis, and yet, options beyond the FOXL2 mutation are still to be identified. Targeting the FOXL2 mutation is likely to be difficult. Hence further targets are potentially needed in order to treat this disease with a more targeted approach. It is clear that other genetic or genomic changes must determine late recurrence or an advanced stage. With a multi-omics approach involving the application of whole genome sequencing, whole-exome sequencing, RNA-seq as well as interrogation of the miRNA-ome, critical driver mutations for GCT or the other SCST will likely be identified, with the hope that these are ‘actionable’ mutations, and thus leading to more precision targeted therapy.

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Author details

Trang Nguyen, Trang Minh Tran, Yee Shen Choo, Maria Alexiadis, Peter J. Fuller and Simon Chu*

Hudson Institute of Medical Research, Clayton, Victoria, Australia

*Address all correspondence to: simon.chu@hudson.org.au

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References

- [1] Scully, R.E., *Classification of human ovarian tumors*. Environ Health Perspect, 1987. **73**: p. 15-25.
- [2] Jamieson, S. and P.J. Fuller, *Molecular pathogenesis of granulosa cell tumors of the ovary*. Endocr Rev, 2012. **33**(1): p. 109-144.
- [3] Kurman RJ, C.M., Herrington CS, Young RH., *WHO Classification of Tumours of Female Reproductive Organs*. 4th ed Lyon: WHO Press, 2014.
- [4] Schumer, S.T. and S.A. Cannistra, *Granulosa cell tumor of the ovary*. J Clin Oncol, 2003. **21**(6): p. 1180-1189.
- [5] Chu, S., et al., *FSH-regulated gene expression profiles in ovarian tumours and normal ovaries*. Mol Hum Reprod, 2002. **8**(5): p. 426-433.
- [6] Amsterdam, A. and N. Selvaraj, *Control of differentiation, transformation, and apoptosis in granulosa cells by oncogenes, oncoviruses, and tumor suppressor genes*. Endocr Rev, 1997. **18**(4): p. 435-461.
- [7] Kitajima, K., Y. Kaji, and K. Sugimura, *Usual and unusual MRI findings of ovarian fibroma: Correlation with pathologic findings*. Magn Reson Med Sci, 2008. **7**(1): p. 43-48.
- [8] Parwate, N.S., et al., *Ovarian fibroma: A Clinico-pathological study of 23 cases with review of literature*. J Obstet Gynaecol India, 2016. **66**(6): p. 460-465.
- [9] Leung, S.W. and P.M. Yuen, *Ovarian fibroma: A review on the clinical characteristics, diagnostic difficulties, and management options of 23 cases*. Gynecol Obstet Invest, 2006. **62**(1): p. 1-6.
- [10] Kim, S.G., Kim, Y.S., Lee, H.J. , *A giant fibroma associated with Meigs' syndrome misdiagnosed as a giant myoma: A case report*. European Journal of Gynaecological Oncology, 2020. **41**(1): p. 134-137.
- [11] Li, X., et al., *Imaging features and pathologic characteristics of ovarian thecoma*. J Comput Assist Tomogr, 2012. **36**(1): p. 46-53.
- [12] Nocito, A.L., et al., *Ovarian thecoma: Clinicopathological analysis of 50 cases*. Ann Diagn Pathol, 2008. **12**(1): p. 12-16.
- [13] Burandt, E. and R.H. Young, *Thecoma of the ovary: A report of 70 cases emphasizing aspects of its histopathology different from those often portrayed and its differential diagnosis*. Am J Surg Pathol, 2014. **38**(8): p. 1023-1032.
- [14] Schultz, K.A., et al., *Ovarian sex cord-stromal tumors*. J Oncol Pract, 2016. **12**(10): p. 940-946.
- [15] Durmus, Y., et al., *Sertoli-Leydig cell tumor of the ovary: Analysis of a single institution database and review of the literature*. J Obstet Gynaecol Res, 2019. **45**(7): p. 1311-1318.
- [16] Nasioudis, D., et al., *Ovarian Sertoli-Leydig and granulosa cell tumor: Comparison of epidemiology and survival outcomes*. Arch Gynecol Obstet, 2020. **302**(2): p. 481-486.
- [17] de Kock, L., et al., *DICER1 mutations are consistently present in moderately and poorly differentiated Sertoli-Leydig cell tumors*. Am J Surg Pathol, 2017. **41**(9): p. 1178-1187.
- [18] Rabban, J.T., A.N. Karnezis, and W.P. Devine, *Practical roles for molecular diagnostic testing in ovarian adult granulosa cell tumour, Sertoli-Leydig cell tumour, microcystic stromal tumour and their mimics*. Histopathology, 2020. **76**(1): p. 11-24.

- [19] Conlon, N., et al., *A survey of DICER1 hotspot mutations in ovarian and testicular sex cord-stromal tumors*. *Mod Pathol*, 2015. **28**(12): p. 1603-12.
- [20] Heravi-Moussavi, A., et al., *Recurrent somatic DICER1 mutations in nonepithelial ovarian cancers*. *N Engl J Med*, 2012. **366**(3): p. 234-242.
- [21] Lim, D. and E. Oliva, *Ovarian sex cord-stromal tumours: An update in recent molecular advances*. *Pathology*, 2018. **50**(2): p. 178-189.
- [22] Schultz, K.A.P., et al., *DICER1-related Sertoli-Leydig cell tumor and gynandroblastoma: Clinical and genetic findings from the international ovarian and testicular stromal tumor registry*. *Gynecol Oncol*, 2017. **147**(3): p. 521-527.
- [23] Yuan, Z., et al., *Clinical characteristics and mutation analyses of ovarian Sertoli-Leydig cell tumors*. *Oncologist*, 2020. **25**(9): p. e1396-e1405.
- [24] McCluggage, W.G., et al., *Gynandroblastoma of ovary with juvenile granulosa cell component and heterologous intestinal type glands*. *Histopathology*, 1996. **29**(3): p. 253-257.
- [25] Hwang, S., et al., *Ovarian Gynandroblastoma with a Juvenile Granulosa Cell Tumor Component in a Postmenopausal Woman*. *Diagnostics (Basel)*, 2020. **10**(8).
- [26] Jang, N.R., et al., *Ovarian Gynandroblastoma with a juvenile granulosa cell tumor component in a postmenopausal woman: A case report and literature review*. *J Pathol Transl Med*, 2018. **52**(5): p. 344-348.
- [27] Oparka, R., et al., *The C134W (402 C>G) FOXL2 mutation is absent in ovarian gynandroblastoma: Insights into the genesis of an unusual tumour*. *Histopathology*, 2012. **60**(5): p. 838-842.
- [28] Chivukula, M., et al., *Recurrent gynandroblastoma of ovary-a case report: A molecular and immunohistochemical analysis*. *Int J Gynecol Pathol*, 2007. **26**(1): p. 30-33.
- [29] Wilberger, A. and B. Yang, *Gynandroblastoma with juvenile granulosa cell tumor and concurrent renal cell carcinoma: A case report and review of literature*. *Int J Surg Pathol*, 2015. **23**(5): p. 393-398.
- [30] Zhao, C., et al., *Identification of the most sensitive and robust immunohistochemical markers in different categories of ovarian sex cord-stromal tumors*. *Am J Surg Pathol*, 2009. **33**(3): p. 354-366.
- [31] Young, R.H., et al., *Ovarian sex cord tumor with annular tubules: Review of 74 cases including 27 with Peutz-Jeghers syndrome and four with adenoma malignum of the cervix*. *Cancer*, 1982. **50**(7): p. 1384-1402.
- [32] Hemminki, A., et al., *A serine/threonine kinase gene defective in Peutz-Jeghers syndrome*. *Nature*, 1998. **391**(6663): p. 184-187.
- [33] Hemminki, A., et al., *Localization of a susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis*. *Nat Genet*, 1997. **15**(1): p. 87-90.
- [34] Shackelford, D.B. and R.J. Shaw, *The LKB1-AMPK pathway: Metabolism and growth control in tumour suppression*. *Nat Rev Cancer*, 2009. **9**(8): p. 563-575.
- [35] Yoo, L.I., D.C. Chung, and J. Yuan, *LKB1--a master tumour suppressor of the small intestine and beyond*. *Nat Rev Cancer*, 2002. **2**(7): p. 529-535.
- [36] Kato, N., et al., *The STK11/LKB1 Peutz-Jegher gene is not involved in the pathogenesis of sporadic sex cord-stromal tumors, although loss of heterozygosity at*

19p13.3 indicates other gene alteration in these tumors. *Hum Pathol*, 2004. **35**(9): p. 1101-1104.

[37] Wang, Z.J., et al., *Allele loss and mutation screen at the Peutz-Jeghers (LKB1) locus (19p13.3) in sporadic ovarian tumours*. *Br J Cancer*, 1999. **80**(1-2): p. 70-72.

[38] Clement, P.B., R.H. Young, and R.E. Scully, *Clinical syndromes associated with tumors of the female genital tract*. *Semin Diagn Pathol*, 1991. **8**(4): p. 204-233.

[39] Gell, J.S., et al., *Juvenile granulosa cell tumor in a 13-year-old girl with enchondromatosis (Ollier's disease): A case report*. *J Pediatr Adolesc Gynecol*, 1998. **11**(3): p. 147-150.

[40] Leyva-Carmona, M., M.A. Vazquez-Lopez, and F. Lendinez-Molinos, *Ovarian juvenile granulosa cell tumors in infants*. *J Pediatr Hematol Oncol*, 2009. **31**(4): p. 304-306.

[41] Rietveld, L., et al., *First case of juvenile granulosa cell tumor in an adult with Ollier disease*. *Int J Gynecol Pathol*, 2009. **28**(5): p. 464-467.

[42] Tamimi, H.K. and J.W. Bolen, *Enchondromatosis (Ollier's disease) and ovarian juvenile granulosa cell tumor*. *Cancer*, 1984. **53**(7): p. 1605-1608.

[43] Tanaka, Y., et al., *Ovarian juvenile granulosa cell tumor associated with Maffucci's syndrome*. *Am J Clin Pathol*, 1992. **97**(4): p. 523-527.

[44] Young, R.H., G.R. Dickersin, and R.E. Scully, *Juvenile granulosa cell tumor of the ovary. A clinicopathological analysis of 125 cases*. *Am J Surg Pathol*, 1984. **8**(8): p. 575-596.

[45] Goudie, C., et al., *Paediatric ovarian tumours and their associated cancer susceptibility syndromes*. *J Med Genet*, 2018. **55**(1): p. 1-10.

[46] Pansuriya, T.C., H.M. Kroon, and J.V. Bovee, *Enchondromatosis: Insights on the different subtypes*. *Int J Clin Exp Pathol*, 2010. **3**(6): p. 557-569.

[47] Kumar, A., et al., *Ollier disease: Pathogenesis, diagnosis, and management*. *Orthopedics*, 2015. **38**(6): p. e497-e506.

[48] Grenet, P., et al., *[Dyschondroplasia and ovarian tumor]*. *Ann Pediatr (Paris)*, 1972. **19**(11): p. 759-64.

[49] Jalaeefar, A., et al., *Granulosa cell tumor of the ovary accompanying with Ollier's disease: First case of contralateral presentations*. *J Obstet Gynaecol India*, 2020. **70**(1): p. 81-85.

[50] Xu, H.S., E. Zhong, and J. Rotman, *Juvenile granulosa cell tumor associated with Maffucci syndrome in pregnancy: A case report*. *Clin Imaging*, 2019. **56**: p. 77-80.

[51] Amary, M.F., et al., *Ollier disease and Maffucci syndrome are caused by somatic mosaic mutations of IDH1 and IDH2*. *Nat Genet*, 2011. **43**(12): p. 1262-1265.

[52] Pansuriya, T.C., et al., *Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome*. *Nat Genet*, 2011. **43**(12): p. 1256-1261.

[53] Jin, Y., et al., *Mutant IDH1 dysregulates the differentiation of mesenchymal stem cells in association with gene-specific histone modifications to cartilage- and bone-related genes*. *PLoS One*, 2015. **10**(7): p. e0131998.

[54] Verdegaal, S.H., et al., *Incidence, predictive factors, and prognosis of chondrosarcoma in patients with Ollier disease and Maffucci syndrome: An international multicenter study of 161 patients*. *Oncologist*, 2011. **16**(12): p. 1771-1779.

- [55] Shah, S.P., et al., *Mutation of FOXL2 in granulosa-cell tumors of the ovary*. N Engl J Med, 2009. **360**(26): p. 2719-2729.
- [56] Mayr, D., et al., *Characteristic pattern of genetic aberrations in ovarian granulosa cell tumors*. Mod Pathol, 2002. **15**(9): p. 951-957.
- [57] Lin, Y.S., et al., *Molecular cytogenetics of ovarian granulosa cell tumors by comparative genomic hybridization*. Gynecol Oncol, 2005. **97**(1): p. 68-73.
- [58] King, L.A., et al., *Mitotic count, nuclear atypia, and immunohistochemical determination of Ki-67, c-myc, p21-ras, c-erbB2, and p53 expression in granulosa cell tumors of the ovary: Mitotic count and Ki-67 are indicators of poor prognosis*. Gynecol Oncol, 1996. **61**(2): p. 227-232.
- [59] Miller, B.E., et al., *Parameters of differentiation and proliferation in adult granulosa cell tumors of the ovary*. Cancer Detect Prev, 2001. **25**(1): p. 48-54.
- [60] Villella, J., et al., *Clinical and pathological predictive factors in women with adult-type granulosa cell tumor of the ovary*. Int J Gynecol Pathol, 2007. **26**(2): p. 154-159.
- [61] Caburet, S., et al., *Combined comparative genomic hybridization and transcriptomic analyses of ovarian granulosa cell tumors point to novel candidate driver genes*. BMC Cancer, 2015. **15**: p. 251.
- [62] Benayoun, B.A., et al., *Adult ovarian granulosa cell tumor transcriptomics: Prevalence of FOXL2 target genes misregulation gives insights into the pathogenic mechanism of the p.Cys134Trp somatic mutation*. Oncogene, 2013. **32**(22): p. 2739-2746.
- [63] Alexiadis, M., et al., *Nuclear receptor profiling of ovarian granulosa cell tumors*. Horm Cancer, 2011. **2**(3): p. 157-169.
- [64] Herriges, J.C., et al., *Identification of two 14q32 deletions involving DICER1 associated with the development of DICER1-related tumors*. Eur J Med Genet, 2019. **62**(1): p. 9-14.
- [65] Streblow, R.C., et al., *Imbalances of chromosomes 4, 9, and 12 are recurrent in the thecoma-fibroma group of ovarian stromal tumors*. Cancer Genet Cytogenet, 2007. **178**(2): p. 135-140.
- [66] Gailani, M.R., et al., *Developmental defects in Gorlin syndrome related to a putative tumor suppressor gene on chromosome 9*. Cell, 1992. **69**(1): p. 111-117.
- [67] Tsuji, T., L. Catasus, and J. Prat, *Is loss of heterozygosity at 9q22.3 (PTCH gene) and 19p13.3 (STK11 gene) involved in the pathogenesis of ovarian stromal tumors?* Hum Pathol, 2005. **36**(7): p. 792-796.
- [68] Chen, Y. and G. Struhl, *Dual roles for patched in sequestering and transducing hedgehog*. Cell, 1996. **87**(3): p. 553-563.
- [69] Kalfa, N., et al., *[Juvenile granulosa-cell tumor: clinical and molecular expression]*. Gynecol Obstet Fertil, 2009. **37**(1): p. 33-44.
- [70] Jamieson, S., et al., *The FOXL2 C134W mutation is characteristic of adult granulosa cell tumors of the ovary*. Mod Pathol, 2010. **23**(11): p. 1477-1485.
- [71] Rosario, R., et al., *Adult granulosa cell tumours (GCT): Clinicopathological outcomes including FOXL2 mutational status and expression*. Gynecol Oncol, 2013. **131**(2): p. 325-329.
- [72] Stewart, C.J., et al., *An immunohistochemical and molecular analysis of problematic and unclassified ovarian sex cord-stromal tumors*. Hum Pathol, 2013. **44**(12): p. 2774-2781.

- [73] Kalfa, N., et al., *Activating mutations of the stimulatory g protein in juvenile ovarian granulosa cell tumors: A new prognostic factor?* J Clin Endocrinol Metab, 2006. **91**(5): p. 1842-1847.
- [74] Salpea, P. and C.A. Stratakis, *Carney complex and McCune Albright syndrome: An overview of clinical manifestations and human molecular genetics.* Mol Cell Endocrinol, 2014. **386**(1-2): p. 85-91.
- [75] Bessiere, L., et al., *A hot-spot of In-frame duplications activates the Oncoprotein AKT1 in juvenile granulosa cell tumors.* EBioMedicine, 2015. **2**(5): p. 421-431.
- [76] Auguste, A., et al., *Molecular analyses of juvenile granulosa cell tumors bearing AKT1 mutations provide insights into tumor biology and therapeutic leads.* Hum Mol Genet, 2015. **24**(23): p. 6687-6698.
- [77] Kalfa, N., et al., *Extinction of FOXL2 expression in aggressive ovarian granulosa cell tumors in children.* Fertil Steril, 2007. **87**(4): p. 896-901.
- [78] Caburet, S., et al., *The transcription factor FOXL2: at the crossroads of ovarian physiology and pathology.* Mol Cell Endocrinol, 2012. **356**(1-2): p. 55-64.
- [79] Leung, D.T.H., P.J. Fuller, and S. Chu, *Impact of FOXL2 mutations on signaling in ovarian granulosa cell tumors.* Int J Biochem Cell Biol, 2016. **72**: p. 51-54.
- [80] Rosario, R., P.A. Cohen, and A.N. Shelling, *The role of FOXL2 in the pathogenesis of adult ovarian granulosa cell tumours.* Gynecol Oncol, 2014. **133**(2): p. 382-387.
- [81] Wang, Y., et al., *The oncogenic roles of DICER1 RNase IIIb domain mutations in ovarian Sertoli-Leydig cell tumors.* Neoplasia, 2015. **17**(8): p. 650-660.
- [82] Wells, S.A., Jr., et al., *Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma.* Thyroid, 2015. **25**(6): p. 567-610.
- [83] Alexiadis, M., et al., *Transcriptomic analysis of stage 1 versus advanced adult granulosa cell tumors.* Oncotarget, 2016. **7**(12): p. 14207-14219.
- [84] Cancer Genome Atlas Research, N., *Integrated genomic analyses of ovarian carcinoma.* Nature, 2011. **474**(7353): p. 609-15.
- [85] Alexiadis, M., et al., *Mutational landscape of ovarian adult granulosa cell tumors from whole exome and targeted TERT promoter sequencing.* Mol Cancer Res, 2019. **17**(1): p. 177-185.
- [86] Hillman, R.T., et al., *KMT2D/MLL2 inactivation is associated with recurrence in adult-type granulosa cell tumors of the ovary.* Nat Commun, 2018. **9**(1): p. 2496.
- [87] Zehir, A., et al., *Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients.* Nat Med, 2017. **23**(6): p. 703-713.
- [88] Pilsworth, J.A., et al., *Adult-Type Granulosa Cell Tumor of the Ovary: A FOXL2-Centric Disease.* J Pathol Clin Res, 2021.
- [89] Da Cruz Paula, A., et al., *Genomic profiling of primary and recurrent adult granulosa cell tumors of the ovary.* Mod Pathol, 2020. **33**(8): p. 1606-1617.
- [90] Roze, J., et al., *Whole Genome Analysis of Ovarian Granulosa Cell Tumors Reveals Tumor Heterogeneity and a High-Grade TP53-Specific Subgroup.* Cancers (Basel), 2020. **12**(5).
- [91] Jamieson, S. and P.J. Fuller, *Characterization of the inhibitor of kappaB kinase (IKK) complex in*

granulosa cell tumors of the ovary and granulosa cell tumor-derived cell lines. Horm Cancer, 2013. **4**(5): p. 277-292.

[92] Pilsworth, J.A., et al., *TERT promoter mutation in adult granulosa cell tumor of the ovary.* Mod Pathol, 2018.

[93] Li, Y., et al., *Non-canonical NF-kappaB signalling and ETS1/2 cooperatively drive C250T mutant TERT promoter activation.* Nat Cell Biol, 2015. **17**(10): p. 1327-1338.

[94] Pestana, A., et al., *TERT biology and function in cancer: Beyond immortalisation.* J Mol Endocrinol, 2017. **58**(2): p. R129-R146.

[95] Chu, S., et al., *Transrepression of estrogen receptor beta signaling by nuclear factor-kappaB in ovarian granulosa cells.* Mol Endocrinol, 2004. **18**(8): p. 1919-1928.

[96] Bilandzic, M., et al., *Betaglycan alters NFkappaB-TGFbeta2 cross talk to reduce survival of human granulosa tumor cells.* Mol Endocrinol, 2013. **27**(3): p. 466-479.

[97] Bell, R.J., et al., *Understanding TERT promoter mutations: A common path to immortality.* Mol Cancer Res, 2016. **14**(4): p. 315-323.

[98] Vedanayagam, J., et al., *Cancer-associated mutations in DICER1 RNase IIIa and IIIb domains exert similar effects on miRNA biogenesis.* Nat Commun, 2019. **10**(1): p. 3682.

[99] Robertson, J.C., C.L. Jorcyk, and J.T. Oxford, *DICER1 Syndrome: DICER1 Mutations in Rare Cancers.* Cancers (Basel), 2018. **10**(5).

[100] Rosario, R., C. Blenkiron, and A.N. Shelling, *Comparative study of microRNA regulation on FOXL2 between adult-type and juvenile-type granulosa cell tumours in vitro.* Gynecol Oncol, 2013. **129**(1): p. 209-215.

[101] McClelland, M.L., et al., *CCAT1 is an enhancer-templated RNA that predicts BET sensitivity in colorectal cancer.* J Clin Invest, 2016. **126**(2): p. 639-652.

[102] Saghatelian, A. and J.P. Couso, *Discovery and characterization of smORF-encoded bioactive polypeptides.* Nat Chem Biol, 2015. **11**(12): p. 909-916.

[103] Steinmetz, R., et al., *Mechanisms regulating the constitutive activation of the extracellular signal-regulated kinase (ERK) signaling pathway in ovarian cancer and the effect of ribonucleic acid interference for ERK1/2 on cancer cell proliferation.* Mol Endocrinol, 2004. **18**(10): p. 2570-2582.

[104] Rosario, R., et al., *The transcriptional targets of mutant FOXL2 in granulosa cell tumours.* PLoS One, 2012. **7**(9): p. e46270.

[105] Chan-Penebre, E., et al., *Selective killing of SMARCA2- and SMARCA4-deficient small cell carcinoma of the ovary, hypercalcemic type cells by inhibition of EZH2: In vitro and In vivo preclinical models.* Mol Cancer Ther, 2017. **16**(5): p. 850-860.

[106] Chen, H., et al., *RUNX3 Promotes the Tumorigenic Phenotype in KGN, a Human Granulosa Cell Tumor-Derived Cell Line.* Int J Mol Sci, 2019. **20**(14).

[107] Karnezis, A.N., et al., *Re-assigning the histologic identities of COV434 and TOV-112D ovarian cancer cell lines.* Gynecol Oncol, 2021. **160**(2): p. 568-578.

[108] Ulbright, T.M., et al., *Poorly differentiated (small cell) carcinoma of the ovary in young women: Evidence supporting a germ cell origin.* Hum Pathol, 1987. **18**(2): p. 175-184.

[109] Foulkes, W.D., et al., *No small surprise - small cell carcinoma of the ovary, hypercalcaemic type, is a malignant*

rhabdoid tumour. J Pathol, 2014. **233**(3): p. 209-214.

[110] Liu, Z., et al., *FOXO1/3 and PTEN depletion in granulosa cells promotes ovarian granulosa cell tumor development*. Mol Endocrinol, 2015. **29**(7): p. 1006-1024.

[111] Bittinger, S., M. Alexiadis, and P.J. Fuller, *Expression status and mutational analysis of the PTEN and P13K subunit genes in ovarian granulosa cell tumors*. Int J Gynecol Cancer, 2009. **19**(3): p. 339-342.

[112] Lague, M.N., et al., *Synergistic effects of Pten loss and WNT/CTNNB1 signaling pathway activation in ovarian granulosa cell tumor development and progression*. Carcinogenesis, 2008. **29**(11): p. 2062-2072.

[113] Boerboom, D., et al., *Misregulated Wnt/beta-catenin signaling leads to ovarian granulosa cell tumor development*. Cancer Res, 2005. **65**(20): p. 9206-9215.

[114] Ohishi, Y., et al., *Nuclear localization of E-cadherin but not beta-catenin in human ovarian granulosa cell tumours and normal ovarian follicles and ovarian stroma*. Histopathology, 2011. **58**(3): p. 423-432.

[115] De Cian, M.C., et al., *Amplification of R-spondin1 signaling induces granulosa cell fate defects and cancers in mouse adult ovary*. Oncogene, 2017. **36**(2): p. 208-218.

[116] Gao, Y., et al., *Constitutively active transforming growth factor beta receptor 1 in the mouse ovary promotes tumorigenesis*. Oncotarget, 2016. **7**(27): p. 40904-40918.

[117] Matzuk, M.M., et al., *Alpha-inhibin is a tumour-suppressor gene with gonadal specificity in mice*. Nature, 1992. **360**(6402): p. 313-319.

[118] Kumar, T.R., Y. Wang, and M.M. Matzuk, *Gonadotropins are essential*

modifier factors for gonadal tumor development in inhibin-deficient mice. Endocrinology, 1996. **137**(10): p. 4210-4216.

[119] Matzuk, M.M., et al., *Development of cancer cachexia-like syndrome and adrenal tumors in inhibin-deficient mice*. Proc Natl Acad Sci U S A, 1994. **91**(19): p. 8817-8821.

[120] Mankanji, Y., et al., *Inhibin at 90: From discovery to clinical application, a historical review*. Endocr Rev, 2014. **35**(5): p. 747-794.

[121] Kim, S.Y., *Insights into granulosa cell tumors using spontaneous or genetically engineered mouse models*. Clin Exp Reprod Med, 2016. **43**(1): p. 1-8.

[122] Li, Q., et al., *SMAD3 regulates gonadal tumorigenesis*. Mol Endocrinol, 2007. **21**(10): p. 2472-2486.

[123] Looyenga, B.D. and G.D. Hammer, *Genetic removal of Smad3 from inhibin-null mice attenuates tumor progression by uncoupling extracellular mitogenic signals from the cell cycle machinery*. Mol Endocrinol, 2007. **21**(10): p. 2440-2457.

[124] Kottarathil, V.D., et al., *Recent advances in granulosa cell tumor ovary: A review*. Indian J Surg Oncol, 2013. **4**(1): p. 37-47.

[125] Pectasides, D., N. Alevizakos, and A.E. Athanassiou, *Cisplatin-containing regimen in advanced or recurrent granulosa cell tumours of the ovary*. Ann Oncol, 1992. **3**(4): p. 316-318.

[126] Uygun, K., et al., *Clinical parameters and treatment results in recurrent granulosa cell tumor of the ovary*. Gynecol Oncol, 2003. **88**(3): p. 400-3.

[127] Zambetti, M., et al., *Cis-platinum/vinblastine/bleomycin combination chemotherapy in advanced or recurrent granulosa cell tumors of the ovary*. Gynecol Oncol, 1990. **36**(3): p. 317-320.

- [128] Homesley, H.D., et al., *Bleomycin, etoposide, and cisplatin combination therapy of ovarian granulosa cell tumors and other stromal malignancies: A gynecologic Oncology group study.* Gynecol Oncol, 1999. **72**(2): p. 131-137.
- [129] Pautier, P., et al., *Combination of bleomycin, etoposide, and cisplatin for the treatment of advanced ovarian granulosa cell tumors.* Int J Gynecol Cancer, 2008. **18**(3): p. 446-452.
- [130] Seagle, B.L., et al., *Ovarian granulosa cell tumor: A National Cancer Database study.* Gynecol Oncol, 2017. **146**(2): p. 285-291.
- [131] van Meurs, H.S., et al., *Hormone therapy in ovarian granulosa cell tumors: A systematic review.* Gynecol Oncol, 2014. **134**(1): p. 196-205.
- [132] Yang, A.D., Curtin, J. & Muggia, F., *Ovarian adult-type granulosa cell tumor: Focusing on endocrine-based therapies.* Int J Endocr Oncol, 2018. **5**(2): p. IJE08.
- [133] Isaacs, R., G. Forgeson, and S. Allan, *Progestagens for granulosa cell tumours of the ovary.* Br J Cancer, 1992. **65**(1): p. 140.
- [134] Malik, S.T. and M.L. Slevin, *Medroxyprogesterone acetate (MPA) in advanced granulosa cell tumours of the ovary--a new therapeutic approach?* Br J Cancer, 1991. **63**(3): p. 410-411.
- [135] Farkkila, A., et al., *Vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 are highly expressed in ovarian granulosa cell tumors.* Eur J Endocrinol, 2011. **164**(1): p. 115-122.
- [136] Farkkila, A., et al., *Serum vascular endothelial growth factor a (VEGF) is elevated in patients with ovarian granulosa cell tumor (GCT), and VEGF inhibition by bevacizumab induces apoptosis in GCT in vitro.* J Clin Endocrinol Metab, 2011. **96**(12): p. E1973-E1981.
- [137] Tao, X., et al., *Anti-angiogenesis therapy with bevacizumab for patients with ovarian granulosa cell tumors.* Gynecol Oncol, 2009. **114**(3): p. 431-436.
- [138] Kesterson, J.P., P. Mhawech-Fauceglia, and S. Lele, *The use of bevacizumab in refractory ovarian granulosa-cell carcinoma with symptomatic relief of ascites: A case report.* Gynecol Oncol, 2008. **111**(3): p. 527-529.
- [139] Brown, J., et al., *Efficacy and safety of bevacizumab in recurrent sex cord-stromal ovarian tumors: Results of a phase 2 trial of the gynecologic Oncology group.* Cancer, 2014. **120**(3): p. 344-351.
- [140] Chu, S., M. Alexiadis, and P.J. Fuller, *Expression, mutational analysis and in vitro response of imatinib mesylate and nilotinib target genes in ovarian granulosa cell tumors.* Gynecol Oncol, 2008. **108**(1): p. 182-190.
- [141] Jamieson, S. and P.J. Fuller, *Tyrosine kinase inhibitors as potential therapeutic agents in the treatment of granulosa cell tumors of the ovary.* Int J Gynecol Cancer, 2015. **25**(7): p. 1224-1231.
- [142] T.J., H., et al., *PD-1, PD-L1 expression in 1599 gynecological cancers: Implications for immunotherapy.* Gynecol Oncol., 2015. **137**: p. 204-205.
- [143] Pierini, S., et al., *Ovarian granulosa cell tumor characterization identifies FOXL2 as an immunotherapeutic target.* JCI Insight, 2020. **5**(16).
- [144] Baldwin, A.S., Jr., *The NF-kappa B and I kappa B proteins: New discoveries and insights.* Annu Rev Immunol, 1996. **14**: p. 649-683.
- [145] Fulda, S., *Smac mimetics as IAP antagonists.* Semin Cell Dev Biol, 2015. **39**: p. 132-138.

[146] Fulda, S., *Smac mimetics to therapeutically target IAP proteins in Cancer*. Int Rev Cell Mol Biol, 2017. **330**: p. 157-169.

[147] Fulda, S. and D. Vucic, *Targeting IAP proteins for therapeutic intervention in cancer*. Nat Rev Drug Discov, 2012. **11**(2): p. 109-124.

[148] Berthelet, J. and L. Dubrez, *Regulation of apoptosis by inhibitors of apoptosis (IAPs)*. Cells, 2013. **2**(1): p. 163-187.

[149] Vaux, D.L. and J. Silke, *IAPs, RINGs and ubiquitylation*. Nat Rev Mol Cell Biol, 2005. **6**(4): p. 287-297.

[150] Leung, D.T.H., et al., *Combined PPARgamma activation and XIAP inhibition as a potential therapeutic strategy for ovarian granulosa cell tumors*. Mol Cancer Ther, 2019. **18**(2): p. 364-375.

[151] Leung, D.T.H., et al., *Targeting XIAP and PPARgamma in granulosa cell tumors alters metabolic signaling*. J Proteome Res, 2019.