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Pathogenesis and treatment of adult-type granulosa cell tumor of the ovary

Anniina Färkkilä,a,b Ulla-Majia Haltiaa,b Johanna Tappera, Melissa K. McConechyc, David G. Huntsmand,e and Markku Heikinheimob,f

Abstract

Adult-type granulosa cell tumor is a clinically and molecularly unique subtype of ovarian cancer. These tumors originate from the sex cord stromal cells of the ovary and represent 3–5% of all ovarian cancers. The majority of adult-type granulosa cell tumors are diagnosed at an early stage with an indolent prognosis. Surgery is the cornerstone for the treatment of both primary and relapsed tumor, while chemotherapy is applied only for advanced or non-resectable cases. Tumor stage is the only factor consistently associated with prognosis. However, every third of the patients relapse, typically in 4–7 years from diagnosis, leading to death in 50% of these patients. Anti-Müllerian Hormone and inhibin B are currently the most accurate circulating biomarkers. Adult-type granulosa cell tumors are molecularly characterized by a pathognomonic somatic missense point mutation 402C>G (C134W) in the transcription factor FOXL2. The FOXL2 402C>G mutation leads to increased proliferation and survival of granulosa cells, and promotes hormonal changes. Histological diagnosis of adult-type granulosa cell tumor is challenging, therefore testing for the FOXL2 mutation is crucial for differential diagnosis. Large international collaborations utilizing molecularly defined cohorts are essential to improve and validate new treatment strategies for patients with high-risk or relapsed adult-type granulosa cell tumor.

Key messages:
• Adult-type granulosa cell tumor is a unique ovarian cancer with an indolent, albeit unpredictable disease course.
• Adult-type granulosa cell tumors harbor a pathognomonic somatic missense mutation in transcription factor FOXL2.
• The key challenges in the treatment of patients with adult-type granulosa cell tumor lie in the identification and management of patients with high-risk or relapsed disease.

Introduction

Granulosa cell tumour (GCT) of the ovary is a rare subtype of ovarian cancer originating from the sex-cord stromal component of the ovary (1). The incidence of GCTs is 0.6–0.8/100,000, and it accounts for 3–5% of all ovarian malignancies (2). GCTs present with two clinically and molecularly distinct subtypes; the juvenile and the adult type (1). Adult-type GCT (AGCT) is the more common, whereas the juvenile comprises only 5% of all GCTs. Juvenile GCT is generally diagnosed at early stage in children and adolescents, and presents with a favorable prognosis, although at advanced stages the clinical course may be more aggressive (3). Although extremely uncommon, the juvenile type can be found in adults and the adult type in children. AGCTs are usually diagnosed in perimenopausal women between ages 50 and 54, although it can occur throughout the adult woman’s lifespan. AGCT is a unique subtype of ovarian cancer with distinct clinical and molecular features. This review focuses on the current view on the pathogenesis and treatment of AGCTs of the ovary.

Presentation and diagnosis

Clinical presentation

AGCTs are clinically distinct ovarian cancers due to their prominent hormonal activity and production of
estrogen and inhibins (1). The most common presenting symptoms are abnormal vaginal bleeding (45%), and abdominal pain or bloating (10–20%) (4). In premenopausal patients, AGCT typically cause irregular bleeding, amenorrhea and more infrequently infertility (4). In postmenopausal patients, abnormal vaginal bleeding associated with a unilateral ovarian mass is the most common clinical presentation. Ascites is rarely present in the primary diagnosis. In 8–15% of the cases, the tumor presents with a spontaneous rupture manifesting as acute abdominal pain and hemoperitoneum (4,5). In ultrasonography, AGCT characteristically presents with a cystic to solid ovarian mass with high vasculature (6). Typically, these patients also have an abnormally thick endometrium, pathological examination may reveal endometrial hyperplasia in 26–38% and synchronous endometrial cancer is diagnosed in 6–7% of the patients (4,7).

**Prognosis**

**Outcomes**

Due to the slow growth and distinguishable hormonal symptoms, AGCTs are generally diagnosed at an early stage. The majority (50–80%) are diagnosed at stage Ia (4,5,8,9), in which the tumor is completely inside the ovarian capsule, and no tumor cells are found outside the ovary (10). The second most common (~30%) is stage Ic, in which tumor cells infiltrate through the ovarian capsule, or the capsule is ruptured either spontaneously or due to iatrogenic reasons. Generally, AGCTs have a remarkably indolent behavior compared to other ovarian malignancies. However, the reported outcomes have significantly varied between cohorts; the reported 5-year overall survival rates between 61% and 95% and disease-specific survival rates between 67% and 99% have been reported (Table 1). Likewise, the recurrence rates may vary between 6% and 48%. However, in a validated large cohort, patients have excellent outcomes, with the overall survival comparable to a general age-matched population; the 5- and 10-year overall survival rates are 98% and 84%, respectively (11). In addition, in advanced stage (III–IV), molecularly defined AGCTs are extremely uncommon.

Even though most early stage AGCT patients experience an excellent outcome, every third patient will eventually develop a tumor relapse, and this will lead to death in 50–80% of the relapsed patients (4,11,12). Thus, a unique challenge in the treatment of AGCT patients lies in the long latency to disease relapse; the typical time to relapse varies between 4 and 8 years (4,8,13) the median being 7.2 years in a histologically and molecularly defined large AGCT cohorts (11). AGCT may, however relapse even after 30–40 years from diagnosis (14), warranting for life-long surveillance of especially in the high-risk patients. The cellular mechanisms of latency and late relapse have not been established in AGCTs, however they may include mechanisms of tumor dormancy similarly to those reported in breast cancer (15).

**Prognostic factors**

Multiple clinical and histological prognostic factors have been investigated for their role in AGCT prognostics, however the results have been inconclusive and varied significantly between cohorts. Tumor stage is the only consistent factor that has been linked to tumor relapse and survival (4,8,16). Among the stage I patients, those with tumor penetrating the ovarian capsule or tumor rupture (stage Ic) have a significantly increased risk of disease relapse (9,17). Consistently, rupture of the tumor capsule has been implicated as an adverse prognostic indicator, also in stage I AGCTs (17–19). Interestingly, in a recent study of verified AGCTs, there was no difference in the risk of relapse between surgically or spontaneously ruptured tumors (17). The identification of risk factors among stage I patients is the clinically most important determinant since stage I patients form the majority of AGCT patients. Thus, future clinical and scientific efforts need to be concentrated on how to identify and manage early-stage AGCT patients at increased risk of relapse. In addition, some studies have reported postoperative residual tumor (19) as well as large tumor size as an adverse prognostic factors (8,18). However, the adjustment of tumor stage is not clearly defined in these studies, and some studies show inconsistent results (12,13,20). Further, it is not clear whether patient age at diagnosis affects the prognosis (4,20), whereas parity and reproductive status do not seem to influence outcome of AGCTs (4).

Regarding histopathological prognostic factors, the different histological subtypes have not been associated with prognosis (19), whereas high mitotic activity and nuclear atypia have been shown to predict worse prognosis (21,22). Multiple histological prognostic factors, e.g. Ki67, p53, members of the epidermal growth factor receptor (EGFR) family, and transcription factor GATA4 have been evaluated (22,23), but their clinical applicability remains unclear. The potential histopathological prognostic factors have not yet been validated in independent validated cohorts limiting their use in clinical decision-making.
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A PubMed search with "granulosa cell tumor prognosis" was performed in 5/2016 and articles in English were evaluated based on the title and abstract. Studies in which the histology of the cases had been reviewed and that reported the survival (OS or DSS) or recurrence of patients with only adult ovarian granulosa cell tumors were included. Abbreviations: #; number, pub; publication, mo; months, DSS; disease-specific survival, OS; overall survival, dg; diagnosis, ND; not defined, pt; patients, yr; year.

aEnd time estimated.
bReported as recurrent and metastatic combined.
cMean (range).
dHistological review in the treating hospital, not for the study.
eRelapse-rate in the Finnish, population-based cohort of 165 AGCTs.
fReported as reverse Kaplan–Meier.

Granulosa and Granulosa- Theca cell tumors

Only stage I disease

Median OS 117 Mo (range 19-334)
**Accurate diagnosis**

AGCTs are relatively uncommon and can show histomorphological patterns similar to a variety of other unrelated tumors, therefore an unequivocal diagnosis of AGCT can be challenging. Consequently, in historical series false positive diagnosis rates of up to 36% have been recorded (4,12). A single somatic missense mutation (C134W) in the gene encoding for transcription factor FOXL2 has been found to be present in ~95% of AGCTs, making it a pathognomonic defining feature (24), and this finding has been verified in multiple independent cohorts (11,25–29). The mutation is specific to AGCT, and is not present in any other tumors including juvenile GCTs (30,31). Thus, the FOXL2 mutation has been shown to be essential in the differential diagnosis of AGCTs (32,33). In conjunction with expert pathological morphologic assessment, FOXL2 mutation testing, and the appropriate immunohistochemical markers is strongly recommended for current clinical pathological diagnoses (11,32,34). As direct Sanger sequencing for the FOXL2 mutation, especially from formalin the fixed paraffin-embedded tissue can often result in false negative results (32), the mutational analysis is recommended to be performed using an allelic discrimination assay or other sensitive methods (11). An accurate diagnosis is crucial since misdiagnosis can have profound effects on patient prognosis; the majority of misdiagnosed cases have been shown to be more aggressive tumors with significantly worse outcomes (11). Conversely, molecularly defined AGCTs present with a consistently indolent course with slow progression. Incorporating FOXL2 mutation testing into routine pathological assessment will aid in moving towards a universal and reproducible correct diagnoses, and create uncontaminated cohorts for clinical trials.

Due to recent technical developments, the specific FOXL2 mutation offers an intriguing opportunity for non-invasive diagnostics from cell-free circulating tumor DNA (ctDNA) (35). The advances in digital droplet PCR technology has allowed for the development of a sensitive and specific technique to identify low-frequency FOXL2 ctDNA mutations in a high background of wild-type DNA (36). According to this recent proof of principle study, the FOXL2 mutation was found in the ctDNA from 35% of patients with primary AGCT, and 19% of patients with relapsed disease. Consistent with previous findings (37), the mutation was more likely found from the plasma of patients with larger tumors (36). The question arises whether ctDNA FOXL2 mutation positivity at primary diagnosis or during follow-up can predict tumor recurrence, similar to findings in patients with early-stage breast cancer (37). However, the clinical utility of ctDNA FOXL2 mutation in AGCT patients will need to be further developed and validated in future clinical trials.

**Pathogenesis**

**Molecular pathogenesis**

The transcription factor FOXL2 plays a crucial role in regulating follicular development in the normal ovary (38), and it is crucial for granulosa cell differentiation (39). In AGCTs, the mechanism how the FOXL2 mutation causes tumor formation remains an area of active investigation. At the molecular level, the C134W mutation is not linked to alterations in the protein structure (40), but instead it causes a change in the posttranslational modification (ubiquitination) leading to impaired interactions of FOXL2 with other transcription factors (41). The current evidence suggests that a key event in AGCT pathogenesis is a failure of the mutant FOXL2 to form specific protein–protein interactions leading to subtle changes in the transcription of target genes (42). This creates a transcriptomics profile portraying the typical hallmarks of cancer (43). The impact of the FOXL2 mutation in AGCT pathogenesis is depicted in Figure 1.

**Interplay of transcription factors**

A key result of the FOXL2 mutation seems to be in the dysregulation of cell cycle and apoptosis. Wild-type FOXL2 negatively regulates cell-cycle progression (44) and promotes apoptosis (45). In contrast, the FOXL2 C134W mutant upregulates genes involved in cell-cycle progression and downregulates genes involved in apoptosis (43,45,46). Mutant FOXL2 has been linked to altered interactions with the SMAD transcription factors of the TGB-β/BMP signaling pathway (40,47), which are essential intracellular mediators in normal granulosa cells. A key effect of FOXL2 appears to be mediated through its interaction with SMAD3 (48,49). In normal granulosa cells, SMAD3 mediates activin A and transforming growth factor-β (TGF-β) signals regulating the expression of cyclin D2 and gonadal genes such as inhibin (50). In AGCTs, SMAD3 has been shown to differentially regulate a gonandotrophin releasing hormone activating promoter together with mutant FOXL2 as compared to wild-type FOXL2 (40). Furthermore, mutant FOXL2 has been shown to inhibit the induction of anti-proliferative follistatin, leading to increased cell proliferation and tumor formation (51).

In addition to SMAD3, FOXL2 interacts with multiple transcription partners in AGCTs. GATA4 is a zinc finger
transcription factor that plays a crucial role in ovarian follicular development granulosa cells being the major site of GATA4 expression in the ovary (52). In granulosa cells, follicle stimulating hormone (FSH) and TGF-β upregulate the expression of GATA4 (53), and GATA4 subsequently acts to moderate many factors crucial for granulosa cell proliferation and function (53), including AMH (54), aromatase (55), and α-inhibin (54). The majority of AGCTs express GATA4 at levels comparable to normal preovulatory granulosa cells (52), and high GATA4 expression in these tumors predicts both increased risk of recurrence and shorter disease specific survival (22). Further, GATA4 protein expression correlates with the expressions of the intrinsic apoptotic pathway inhibitor Bcl2, and proproliferative cyclin D2 (56), and overexpression of GATA4 protects AGCT cells from apoptosis (57). GATA4 has also been shown to physically interact with SMAD3 and FOXL2 to modulate gene expression, cell viability, and apoptosis in AGCTs (58). Taken together, these data suggest that GATA4 acts as an anti-apoptotic factor promoting tumor growth in AGCTs. Although FOXL2 and FOXO2, and GATA4 play pivotal roles in AGCT tumorigenesis, they are difficult to target when developing new treatment strategies for advanced AGCT. Therefore, future therapeutic agents may need to be targeted at the downstream effects of these transcription factors, most importantly factors related to apoptosis, proliferation and hormonal regulation.

**Hormonal factors**

AGCTs are derived from the rapidly dividing granulosa cells of small preantral follicles, and express the receptors for several hormonal regulators (59). Wild-type FOXL2 regulates the expression of multiple hormonal factors such as SF1, CYP19A1, HSD17B1, ESR1, ALK6 (BMPR1beta), SOX9, and STAR (60,61). Wild-type FOXL2 causes the repression of STAR and an increase in CYP19A1 maintaining the female phenotype in granulosa cells (39). In granulosa cells, follicle-stimulating hormone (FSH) is an integral regulator of proliferation and hormonal function. FSH binds to its transmembrane receptor, activates transcription via cAMP-mediated pathways resulting in increased expression of, e.g. CYP19A1 (aromatase) and cyclin D2, and promoting cell proliferation. AGCTs are known to express functional FSH receptors activating FSH signaling (62). At diagnosis, AGCT patients typically present with low-circulating FSH levels due to inhibitory feed-back mechanisms of tumor-produced estradiol and inhibins. The pathogenetic role of FSH in AGCTs remains inconclusive, and several studies have failed to identify genetic alterations in FSH receptor, or other G-protein coupled receptors (59,62). In addition to FSH, FOXL2 regulates aromatase expression, and mutant FOXL2 has been shown to directly activate the aromatase promoter (63). AGCT cells have abundant aromatase activity (64), and consequently AGCTs produce estrogens (65). AGCTs also express high levels of estrogen receptors; nuclear estrogen receptor (ER) α expression has been reported in a minority (20%) of AGTS, whereas virtually all AGCTs are ERβ positive (66). In addition, AGCTs have been reported to express a newly characterized, membrane-bound G-protein linked estrogen receptor (GPER1 or GPR30) (67). The para/autocrine actions of tumor-secreted estradiol are unknown, and evidence supporting the assumption that endogenous estradiol promotes tumor growth is lacking. By contrast, it has been shown that estradiol binding to nuclear estrogen receptors (ERα or ERβ) does not activate transcription in AGCT cells due to repression by the NFkB pathway via SMAD3 (68,69). In addition, recent evidence suggests that estradiol may in fact decrease metastasis and invasion of AGCT cells via activation of GPER1 (67). The effects of tumor-produced and exogenous estradiol, such as hormone replacement therapy, need to be further explored. This is also vital in order to interrogate the mechanism of action of the currently applicable hormonal treatments, such as aromatase inhibitors. A recent single-institute retrospective study reported only modest responses with hormone therapy (objective response rate 18%), although a significant proportion (60%) of the patients remained with stable disease (70). Future studies will hopefully shed more light into whether the hormonal pathways can also be exploited to prevent relapse in high-risk patients similar to other hormone-dependent cancers.

**TGF-β family members**

Anti-müllerian hormone (AMH), also referred to as Müllerian inhibiting substance, belongs to the TGF-β/bone morphogenetic protein family of signaling molecules. AMH is a key regulator of follicular development in the ovary (71). In granulosa cells, AMH expression is regulated by steroidogenic factor-1, GATA4 (72), and FSH (73), and the granulosa cell-produced AMH further regulates follicular development in the ovary (71). AMH exerts its effects by binding to a heterodimeric cell surface receptor complex consisting of type I and type II (AMHRII) receptors, that activate downstream SMAD 1, 5, and 8. In the adult female, AMHRII is expressed in granulosa cells and at low levels in ovarian stroma and endometrium (74). In gynecological
In the treatment of primary AGCT, complete surgical removal of the tumor, uterus, ovaries and fallopian tubes, complemented with staging procedures (peritoneal washings, biopsies, and infracolic omentectomy) form the golden standard (82). Pelvic and para-aortic lymphadenectomy (i.e., surgical removal of lymph nodes) are generally not recommended (82), and only bulky or suspicious nodes should be removed. Surgery can be performed in either laparotomy or laparoscopy (33% of primary operations), while the laparoscopic approach has been reported safe and associates with less morbidity (83). In patients presenting with a local disease (stage Ia) and wishing to preserve their fertility, a conservative surgery sparing the normal contralateral ovary and uterus can be performed. In these cases, however, a careful staging procedure coupled with endometrial sampling should be carried out to exclude metastatic disease and/or concurrent endometrial pathology. It is noteworthy, that there is no consensus of whether radical surgery should be performed when these patients have completed their childbearing or when they reach menopause.

The relapsed disease is often multifocal, and complicated by the fact that there is no standard surgical approach or treatment protocol. The most common site of relapse is the pelvis, but abdominal and peritoneal disease, as well as retroperitoneal metastases can occur (5,83,84). Similar to primary disease, the treatment of relapsed disease should aim for optimal surgical debulking whenever possible (5,82,84). This is crucial since the presence of postoperative residual tumor has been shown to be an important prognostic factor for both subsequent tumor relapse, and survival (8,13). Further, patients with recurrent disease are likely to benefit even from repeated surgical approaches if optimal debulking can be achieved (5). And indeed, according to two retrospective reports, an optimal debulking with no macroscopic residual tumor can be achieved in the majority of the patients in secondary surgery (83,84). However, multivisceral surgical procedures may be needed to achieve optimal debulking, which associate with a significantly increased risk of postoperative complications. However, a prolonged disease-free survival was reported in a small patient series regardless of the additional risks associated with repeated and extensive surgical procedures (85).

**Chemotherapy**

Surgery forms the cornerstone of treatment in most AGCTs that are diagnosed at an early stage, and no medical therapies are usually considered. Advanced and/or inoperable chemotherapy is offered, although its efficacy and prognostic significance largely remain unsure. The role of adjuvant chemotherapy in AGCTs...
is also obscure; reasonably high response rates have been reported (86,87), however, adjuvant chemotherapy does not seem to significantly affect patient outcomes (8). Traditionally, platinum-based combination therapies have been utilized as the primary therapeutic option (88,89). Response rates for the most common combination of bleomycin, etoposide and cisplatin vary from 37% to 83% in older studies (88,90), but in the most recent series the responses are only moderate, reaching 22–35% (9,89). It must be noted, however, that the current evidence is based on mostly retrospective studies on non-validated AGCT cohorts, presenting as a potential confounder when evaluating these responses. Combination chemotherapy with paclitaxel and carboplatin has also been used, providing with the same efficacy albeit less toxicity compared with bleomycin, etoposide and cisplatin (90). Interestingly, The Gynecologic Oncology Group is currently running a randomized trial comparing these two combination treatments, hopefully providing a clarification to the choice of optimal chemotherapeutic regimen.

**Hormonal and targeted treatments**

AGCTs are known to express steroid hormone receptors and produce estradiol. Therefore, hormonal treatments have been empirically utilized in AGCTs, mostly as a last resort in patients with non-operable AGCTs. Hormonal therapies have also been considered if the patient has not tolerated or the tumor has not responded to conventional chemotherapy. The treatment modalities have included progestins, gonadotrophin releasing hormone agonist, selective estrogen receptor modulators, and aromatase inhibition. In a systematic review including 19 studies describing the response to hormonal therapy, the pooled objective response rate was as high as 71% (91). However, in a retrospective analysis of 22 patients from a single institute, the objective response rate was unexpectedly low (18%) (70). Similarly, Wilson et al. reported a response rate of 14% to aromatase inhibitors in patients with relapsed stage I AGCTs (9). It is noteworthy, that no randomized trials have been performed regarding hormonal treatments, and the current literature consists only relatively small retrospective series and case reports. In addition, none of these studies have been performed in FOXL2 molecularly defined cohorts, and there is potential bias from patient selection and response evaluation for the benefit of these treatments. Thus, the true efficacy of hormonal therapy in AGCTs remains unknown. Further, the biological principles and mechanisms of action of hormonal treatments in AGCTs are unclear (see above). After the hormonal pathogenesis is clarified in more detail, and there is supportive evidence from a randomized trial, hormonal therapy may be an option in the treatment of AGCTs.

The only targeted therapy with proven efficacy in advanced ovarian cancer is angiogenesis inhibition, most importantly with humanized monoclonal VEGF antibody bevacizumab (92). In AGCTs, bevacizumab was considered active in a phase II clinical trial on advanced AGCTs (93). Thus, bevacizumab may be a viable option in advanced AGCTs.

**Follow-up and timing of treatment**

Follow-up of AGCT patients consists of standard gynecological examinations with ultrasound and blood samples for at least 3–5 years (82,94). The roles of gynecological exam and transvaginal ultrasound in clinical follow-up are unclear as their sensitivity to detect relapses outside the pelvis are limited. Thus, serum markers are commonly utilized as a part of the standard follow-up scheme. Serum inhibin B has the strongest evidence as a tumor marker for AGCTs (95). Granulosa cells of developing follicles produce inhibin B and its serum levels are high in the early follicular phase (96). After menopause, circulating inhibin B concentrations are undetectable. In AGCT patients, inhibin B is superior to inhibin A, with reported sensitivities between 88-100% for inhibin B, and 67-77% for inhibin A, respectively (97). AMH has also been validated as a marker for AGCT, and both AMH and inhibin B are equally sensitive (92 and 93%) and specific (82 and 83%) in this disease (75). In addition, both markers are also elevated in relapsed disease and their levels positively correlate with disease burden (75). The combination of AMH and inhibin B seems to be superior to inhibin B alone in detecting macroscopic disease. In premenopausal patients, there are no established cut-off levels for AMH or inhibin B, and new studies are needed to establish the reference values for AMH and inhibin B. Currently, the levels should be evaluated individually, and patients with rising AMH value that functional granulosa cells are the only source of AMH and inhibin B. It is thus recommended that both markers should be measured at diagnosis, and subsequently one positive marker, either AMH or inhibin B, may be monitored during follow-up (75).

AMH and inhibin B levels have been reported to rise several months or even years before the onset of clinical symptoms of the relapse, the lead-times have been reported 0.9–2.8 years for inhibin B, and 3.4 years for AMH (75,97). The clinical significance of the
early marker rise in regards to patient survival and morbidity can only be speculated upon. To date, there is no evidence supporting the treatment of asymptomatic ovarian cancer patients (98). However, early detection of a relapse by close monitoring with serum markers may result into a more optimal surgical management of the relapse (99).

**Treatment challenges and future perspectives**

The key challenges and suggested solutions in the management of AGCT patients are summarized in Table 2. In support of recent evidence, accurate diagnostics is the cornerstone in the treatment of AGCT patients, and expert pathological review in conjunction with FOXL2 mutation testing should become the standard of care for patients with AGCT.

The surgical treatment of AGCT should aim for optimal cytoreduction, coupled with complete staging, and the centralization of treatment of these patients is essential to ensure the quality and resources of the treatment. The prognostic difference between stage Ia and Ic patients is clinically relevant (17), and special attention should be paid on the surgical management of these patients. In stage Ia patients, rigorous efforts should be concentrated at preventing tumor rupture and dissemination of the tumor cells onto the abdominal cavity during operation. Upon laparoscopic approach, this requires a trained endoscopic surgeon, and the use of special instruments to safely remove the tumor. This underlines the importance of the preoperative evaluation, and the referral of these patients to a gynecological oncologist for primary care.

Further, a major clinical challenge is to identify patients at risk of recurrence. In addition to tumor stage, the current literature offers no consistent prognostic factors. Therefore, further studies in validated cohorts are needed to identify AGCT patients at increased risk of relapse, and to find out whether they would benefit from adjuvant treatments or maintenance therapy to prevent recurrence.

A demanding clinical challenge also arises from the treatment of relapsed or advanced molecularly defined AGCTs. In these cases, platinum-based adjuvant chemotherapy is commonly utilized. However, it is known that platinum-based therapies most efficiently target rapidly dividing cells with DNA repair defects. Further, the rationale of using platinum in AGCTs is based on small, mostly retrospective series, and more importantly from cohorts with no diagnostic validation (89). Recent studies have shown that the patients with the most aggressive AGCTs are more likely to have a different tumor type and are not AGCTs (11), causing potential bias when evaluating the treatment responses from historical cohorts. Notably, AGCTs seem to retain the pathognomonic FOXL2 mutation, and their original gene expression profile and chromosomal architecture even after multiple relapses. The clinical picture of AGCT with slow growth, indolent disease course make studies on new drugs and combinations in prospective clinical trials difficult and time-consuming. Large international clinical trials with molecularly defined AGCT cohorts are needed to validate new treatment strategies for patients with high-risk early-stage and advanced AGCTs. The establishment of new effective treatments should also rely
on the deeper understanding of the pathogenesis of AGCTs.

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Figure 1. AGCT pathogenesis. The central pathogenic FOXL2 mutation 402C > G (C134W) alters gene expression in AGCTs leading to increased proliferation, decreased apoptosis, and characteristic hormonal alterations. In addition, there are multiple intrinsic signaling routes that inhibit the growth of AGCTs. Red arrows up indicate increased expression related to mutant FOXL2, and blue arrows down indicate decreased expression, respectively. Solid arrows indicate activating signal and dashed arrows indicate inhibiting signal. Transcription factors FOXL2 (C134W), GATA4 and SMAD3 co-operatively regulate gene expression in AGCTs. Increased activity of SMAD3 of the TGF-β pathway leads to proliferation and transcriptional changes, while decreased expression of follistatin (FST) increases the pro-tumorigenic Activin signaling, as the expression of its receptor (ACVR) is simultaneously increased. The signal mediators of the growth-inhibitory AMH/BMP-type pathway are decreased in AGCTs. Due to FOXL2 mutation and FSH signaling, the expression of pro-proliferative factor Cyclin D2 is increased in AGCTs contributing to increased proliferation. Apoptosis is decreased in AGCTs both due to decreased expression of pro-apoptotic factors such as Fas and TNFR1, and increased expression of anti-apoptotic factors such as Bcl2. The the hormonal changes associated with AGCTs include expression of aromatase enzyme (CYP19A1) leading to estradiol (E2) production. ER β transcriptional activation is inhibited by increased expression of NFK β and c-Jun, whereas GPER1 activation by E2 seems to decrease migration and invasion in AGCTs via p-ERK1/2. Circulating E2, and inhibit (α chain in the tissue, A/B in the circulation) inhibit GnRH release from the hypothalamus, leading to decreased release of FSH from the pituitary. The hormonal treatments utilized in AGCT include aromatase inhibitor (AI), as well as GnRH analog/antagonists (GnRHi). Abbreviations: AI: Aromatase inhibitor; AMH: Anti-Müllerian hormone; BMP: Bone Morphogenetic Factor; E2: Estradiol; FSH: Follicle stimulating hormone; FST: Follistatin; GnRH: Gonadotrophin Releasing Hormone; NFKβ: Nuclear Factor kappa- β; p-ERK1/2: phosphorylated Extracellular Signal-Regulated Kinases 1/2; TGF-β: Transforming Growth Factor-β; TT: testosterone.

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References


