The new molecular biology of granulosa cell tumors of the ovary

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Abstract

Granulosa cell tumors (GCTs) of the ovary belong to the group of ovarian sex-cord stromal tumors and represent 5 to 10% of ovarian malignancies. GCTs exhibit several morphological, biochemical and hormonal features of normal proliferating pre-ovulatory granulosa cells, such as estrogen biosynthesis. Prognostic factors of this condition are lacking, and alternative treatment options to preserve future fertility are needed. Several groups have shown that two genetic factors implicated in GCTs are of particular interest. The gsp oncogene is a constitutive activating mutation of the Gsα subunit and is correlated with the prognosis of the tumor. FOXL2 is a transcription factor gene involved in ovarian development and function, whose expression is reduced and which is mutated in the majority of GCTs. FOXL2 appears to play a major role in cell cycle regulation. These recent findings open new pathophysiological insights into GCT development as well as revisitation of granulosa cell and ovarian function.

Natural history of granulosa cell tumors of the ovary

Granulosa cell tumors (GCTs) of the ovary are relatively uncommon neoplasms, representing approximately 5 to 10% of all ovarian malignancies [1,2]; the others are germ cell tumors (teratomas and yolk sac tumors, 60 to 70%) and epithelial adenomas (10 to 20%) [3]. GCTs of the ovary belong to the group of ovarian sex-cord stromal tumors. Other tumors in this class are thecoma-fibromas, Sertoli cell tumors, sex cord tumors with annular tubules, and gynandroblastomas. The majority of patients with GCTs are adults, but 5% are pre- or peri-pubertal [4]. Consequently, two histological types of GCTs can be distinguished on a histopathological basis: juvenile and adult. GCTs exhibit several morphological, biochemical and hormonal features of normal proliferating pre-ovulatory granulosa cells, including both estrogen and inhibin biosynthesis [5]. This tumoral hyperestrogenism induces precocious puberty in children (premature breast development, vaginal bleeding) and advanced growth and bone maturation. The prognosis of juvenile GCTs is excellent overall: tumor recurrence and metastasis are rare and usually occur early [6]. On the other hand, adult GCTs are low-grade indolent malignant neoplasms that display a significant propensity for recurrence and metastasis, and they may cause menorrhagia or intermenstrual bleeding. The cornerstone of treatment remains surgery. The disease in young patients is often confined to one ovary and thus, in order to preserve fertility, a unilateral salpingo-oophorectomy is preferable. In the case of postmenopausal women, a total abdominal hysterectomy and bilateral salpingo-oophorectomy should be performed [7].

Challenges in GCT management

Nevertheless, management of patients with ovarian GCTs needs to be improved. First, the recurrent or metastatic tumor may manifest many years after removal of the primary neoplasm, with intervals of 10 or even 20 years being not uncommon, especially in the adult disease [8,9]. Clinical follow-up of these patients is thus critical, but prognostic factors are lacking and assessment of the recurrence risk remains imprecise. For instance, in International Federation of Obstetricians and Gynaecologists (FIGO) stage I disease (that is, intra-ovarian tumor with no extension, the most frequent stage at diagnosis according to FIGO), no association between outcome and the clinical signs or tumor histology (mitotic, nuclear atypia, ploidy, Ki67 expression) has been undisputedly demonstrated [10,11]. Furthermore, in more advanced disease, aggressive debulking surgery and postoperative chemotherapy may be required [8]. Alternative treatment options and the use of molecular markers are thus necessary, especially for young women whose fertility should be preserved.

FOXL2 and GCTs of the ovary

FOXL2 is a recent candidate gene in the pathophysiology of GCTs and could prove useful to evaluate their prognosis.
Indeed, FOXL2 is a winged helix/forkhead transcription factor gene involved in ovarian development and function [12,13]. FOXL2 ovarian expression in mammals starts before the morphological differentiation of the gonad is recognizable, and persists until adulthood, mainly in granulosa cells [14,15]. FOXL2 is thought to be a key factor in the early development and maintenance of the vertebrate ovary [12,15]. Its expression in granulosa cells is suppressed, or at least lowered, in the majority of juvenile GCTs, particularly in those with the most aggressive pattern of progression [16]. Similar expression studies have yet to be performed in adult GCTs.

Interestingly, Shah et al. [17] recently studied four adult GCTs using whole-transcriptome paired-end RNA sequencing. After removing the previously described germline genetic variants and non-specific mutations present in non-GCTs, the authors identified the somatic missense point mutation c.402C→G (p.C134W) in all four specimens. Subsequently, they used direct sequencing, restriction fragment length polymorphism analyses (RFLPs), and a real-time PCR-based allelic discrimination assay to genotype FOXL2 in a large collection of tumors. The p.C134W mutation was found in 97% of 89 additional adult GCTs studied, and was detected in only one case out of eight juvenile GCTs. The mutation was also found in 21% of thecomas, that is, 3 out of 14, in the study of Shah et al. Further studies are needed to determine the link between this mutation and GCTs, and whether this mutation alone is sufficient to induce tumor progression.

Other genetic factors implicated in ovarian GCTs
Genetic defects in other signaling pathways have been investigated in GCTs. Although Peutz-Jeghers syndrome is associated with an increased risk for GCTs, neither allele loss at the disease locus (19p13.3) nor mutations in the LKB1 gene are associated with sporadic GCTs [18,19]. Similarly, despite the association of epithelial ovarian cancer with the familial breast cancer genes BRCA1 and BRCA2, sporadic mutations have not been reported for GCTs.

Other interesting candidate pathways are those involving the mitogen-activated protein kinases [20] and growth factors, because they are known to induce increased oncogene functioning or a loss-of-anti-oncogene function in several solid tumors. However, King et al. [21] failed to identify any prognostic value for the oncogenes c-myc, p21-ras, c-erb B2, and p53 in a group of 40 GCTs. Similarly, the Wilms’ tumor suppressor gene (WT1) and TP53 genes, which play a role in follicular development, have been tested [22]. WT1 is expressed in GCT, but neither mutations nor loss of heterozygosity have been identified [23]. TP53 mutations are frequent in numerous cancers, especially in epithelial tumors of the ovary, but there is neither hyperexpression [24] nor mutation [25] of this gene in GCT.

The role of the follicle-stimulating hormone (FSH) signaling pathway is also strongly suspected in GCTs for two reasons: firstly, normal granulosa cells are under the control of FSH and, secondly, the gene expression profile of GCTs is consistent with a constitutive activation of FSH signaling [26]. However, there is no evidence of activating mutations in the gene encoding the FSH receptor [27-29]. FSH signaling involves the coupling of heterotrimeric G proteins to activate intracellular second messenger systems, mainly the cAMP-dependent protein kinase (PKA).

Conflicting data have been reported regarding the presence of a constitutive activating mutation of the Gαs subunit in GCTs, the so-called gsp oncogene. It has rarely been described in adult GCTs [30,31], but our group has identified it frequently in the juvenile type, where mutations in hot spot position 201 (p.R201C or p.R201H) were found in 30% of patients [32]. The precise role of this mutation in the transformation of ovarian cells into malignant cells remains unclear, but it has been demonstrated in other tissues that the rate of cell proliferation and invasiveness can be influenced by the constitutive activation of Gαs [33]. The oncological stages are indeed significantly different according to the gsp oncogene status. Patients with a hyperactivated Gsa exhibit significantly more advanced tumors, and almost 80% of gsp-positive patients present with an extra-ovarian extension (stage Ic) or have a recurrence [32].

Potential diagnostic and therapeutic applications
The clinical applications of these findings may be promising in the near future, for several reasons. Firstly, identification of a recurrent mutation in FOXL2 may be used as another diagnostic tool for adult GCTs, in addition to the classical pathological and immunohistochemical features. It may be particularly useful to explore granulosa tumor participation in heterogeneous ovarian non-germinal tumors with components of different origins. Also, the low frequency of the FOXL2 mutation in juvenile GCTs compared with adult cases, as reported by Shah and collaborators [17], supports the distinction made between the juvenile type and the adult type based on clinical presentation, pathological features and natural progression [1]. Clearly, differentiating the two types of tumor is clinically relevant, since relapses of adult GCTs are more frequent and may occur later than juvenile GCTs. The mutational or expression status of FOXL2 could thus be of importance in adapting the length of postoperative follow-up. Another genetic abnormality that could be critical in the follow-up of these patients is the gsp oncogene status, especially for juvenile GCTs. A constitutively activated Gsa is indeed involved in the natural history of the tumor and is a prognostic factor of its extension. Finally, the finding of both extinction [16] and mutation [17] of FOXL2 in GCTs highlights the role of this
gene in the regulation of cell proliferation. These findings increase the body of evidence implicating the FOX family genes, whether as oncogenes or anti-oncogenes, in malignant processes such as rhabdomyosarcomas [34], secondary acute myeloblastic leukemia [35], and laryngeal, lung, breast [36-38] and pancreatic cancers [39]. FOXL2 may act as a transcriptional regulator and a coordinator of SMAD3 downstream targets [40,41] that, with SMAD2 and the TGF-β superfamily ligands, regulate granulosa cell proliferation [42]. These recently identified steps could be the targets of the next generation of therapies.

Competing interests
The authors declare that they have no competing interests.

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