Adult-Type Granulosa Cell Tumors and FOXL2 Mutation

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Abstract

Little is known about the pathogenesis of ovarian granulosa cell tumors. Recently, we reported the identification of a somatic FOXL2 402C→G mutation that is present in virtually all adult-type granulosa cell tumors, but not in a wide range of other tumor types. This finding has important implications for the diagnosis and classification of ovarian sex cord-stromal tumors, provides insight into the pathogenesis of adult-type granulosa cell tumors, and opens possibilities for the development of targeted therapies. [Cancer Res 2009;69(24):9160–2]

Granulosa cell tumors (GCT) of the ovary are relatively uncommon (less than 5% of all ovarian malignancies). GCTs can be divided on the basis of histopathological features into juvenile and adult types; these designations reflect the usual age group in which each type occurs. Adult-type GCTs (A-GCT) are most frequently diagnosed in perimenopausal women, at a mean age that is 10 years younger than that of women diagnosed with epithelial ovarian cancer. Patients with A-GCT usually present with low stage disease and have a relatively favorable prognosis, yet the risk of recurrence is unpredictable, and may occur 10 to 30 years after initial diagnosis. For women with recurrent disease or advanced stage tumors at diagnosis, there is no effective treatment beyond surgery, and 20 to 30% of women diagnosed with A-GCT will ultimately die of their disease (1).

Past efforts to understand the molecular pathogenesis of GCTs have been limited by their relative rarity. Since pre-ovulatory growth of normal granulosa cells is induced by follicle-stimulating hormone (FSH), alterations in the FSH pathway were suggested to play an integral role in GCT pathogenesis. However, neither FSH receptor nor associated G-protein mutations have been identified in A-GCTs (2). Mutations in the GNAS gene, which codes for a stimulatory G protein, have been described in a minority of juvenile-type GCTs (J-GCT) but not in A-GCTs or other ovarian cancers (3). In 1989 inhibited was reported as a tumor marker for GCTs (4). Inhibit, however, conveys antigrowth signals by suppressing endogenous FSH levels and is therefore unlikely to be an oncogenic contributor to the etiology of GTC. Oncogenes (MYC, ERBB2) and tumor suppressors (TP53) important in other tumor entities have been investigated in GCT without success. Compared with high-grade serous carcinomas of the ovary, GCTs show very little chromosomal instability. Given this relatively stable genome, we hypothesized that whole transcriptome RNA sequencing might identify one or a small number of pathognomonic genetic alterations through analysis of a limited number of cases of A-GCT.

Using whole-transcriptome paired-end RNA sequencing, we recently reported the identification of a missense point mutation 402C-G (C134W) in FOXL2 a member of the forkhead-winged helix family of transcription factors in all of the four A-GCTs and then showed it to be present in 86 out of 89 (97%) additional cases of A-GCT (5). The mutation was absent in 329 unrelated epithelial ovarian or breast tumors. Distinction between sex cord-stromal tumors and non-sex cord-stromal tumors can be problematic for histopathologists, and the differential diagnosis for A-GCT includes primary or metastatic epithelial cancers, mesenchymal tumors, neuroendocrine tumors, and melanoma. Immunohistochemical markers such as inhibin, calretinin, and WT1 show fairly good sensitivity for A-GTCs (91%, 81%, and 78%, respectively; ref. 6), but they are not completely specific, and there remain cases in which the distinction of A-GCT from non-sex cord-stromal tumors is difficult. In these cases detection of the FOXL2 402C-G mutation could be used to establish a diagnosis of A-GCT.

Within the group of sex cord-stromal tumors there is morphologic and immunophenotypic overlap between different tumor cell types, and no cell marker useful for differential diagnosis within the category of sex cord-stromal tumors has been described. The four major sex cord-stromal cell types are granulosa, Sertoli, steroid, and fibrothecomatous cells (Fig. IA-D). Some sex cord-stromal tumors contain more than one cell type, such as granulosa-theca cell tumors with both granulosa and theca cell components. We reported the presence of the FOXL2 402C-G mutation in 3 out of 14 thecomas, and retrospective evaluation of one of these cases revealed a small granulosa cell component. Thecomas are benign tumors in most cases and it will be interesting to determine whether the rare cases of thecoma that behave in a malignant fashion are those with the FOXL2 402C-G mutation. Pure Sertoli-Leydig cell tumors, steroid cell tumors, and juvenile-type GCTs are not associated with this mutation. Juvenile-type GCTs are of particular interest; they were first described more than 25 years ago as a rare subtype of GCT with distinct morphological features occurring in a different age group (7). FOXL2 expression is reduced or absent in about half of J-GCTs (8), whereas it is highly expressed in A-GCTs (5). With the exception of one case, we did not detect the FOXL2 402C-G mutation in J-GCTs, and we believe that this single positive case may have been a misclassified A-GCT. The absence of the FOXL2 402C-G mutation in J-GCTs supports the view that this is a separate disease from A-GCT. A probable activating mutation in the GNAS gene, which encodes a stimulatory G protein involved in coupling FSH signaling to the intracellular second messenger system (3), has been described in 30% of J-GCTs. There is also a naturally occurring mouse model specifically for J-GCTs (9). Diagnostic testing for the FOXL2 mutation could become widely used, as it can serve to both distinguish A-GCT from both non-sex cord-stromal tumors and other tumors in the sex cord-stromal category. In addition to directly screening for the mutation, mutant specific antibodies could, theoretically, be used to diagnose A-GCT.
As A-GCTs do not respond to standard chemotherapeutic protocols there is a need for new treatments. FOXL2 is a highly conserved transcription factor among species and is expressed as a nuclear protein in the developing eye, pituitary, embryonic and adult ovary, and elsewhere (10). It is not expressed in developing male gonads and therefore is considered to be the earliest sexually dimorphic marker of ovarian determination. Previously, only loss of function mutations were reported for FOXL2 in patients with blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) type I, which includes ovarian failure (11). FOXL2 knockout mice have increased follicular loss and oocyte atresia, indicating an anti-apoptotic role of FOXL2 (12). The FOXL2 402C→G mutation is localized within the forkhead domain (Fig. 1E). In the majority of cases the FOXL2 mutation in A-GCT was heterozygous. In cases homozygous for this mutation, we confirmed that FOXL2 protein was located in the nucleus, corresponding to its function as a transcription factor. Aberrant cytoplasmic localization of FOXL2 protein has been associated with loss of function mutation (13). Although there may be additional changes contributing to initiation and progression of A-GCT, these data suggest that the FOXL2 402C→G leads to a gain or change of function and is the likely "driver" mutation for this cancer type.

To understand the mechanism through which this FOXL2 mutation contributes to the transformation of normal granulosa or sex cord-stromal stem cells, a more comprehensive knowledge about FOXL2 protein function is needed. FOXL2 is a transcription factor; an activating mutation, therefore, might have pleiotropic effects. Some targets of FOXL2 have been identified; e.g., it suppresses steroid synthesis by suppressing expression of StAR (steroidogenic acute regulatory) gene, which controls cholesterol transport from the outer to inner mitochondrial membrane. FOXL2 increases estrogen conversion by inducing the aromatase gene CYP19A1 (14, 15), in keeping with the physiological role of granulosa cells as the major source of estrogen conversion (from androgens derived from theca cells). Deregulated FOXL2 activity might account for the highly characteristic clinical manifestations of hyperestrinism, seen in 70% of patients with A-GCT. Yet it is not clear whether these "physiological" targets or other targets of FOXL2 are involved in tumorigenesis. Modeling the amino acid change (C134W) suggests that it does not directly affect DNA binding by FOXL2, and it is likely that FOXL2 interaction with other protein partners is disrupted (Fig. 1E). Candidates are the Smad transcription factors, the effectors of TGFB, and BMP family signaling (16). FOXL2 has been shown to act as part of an AP-1, Smad3, Smad4 complex to activate transcription of the GnRH receptor in pituitary cells. Many questions remain.

**Figure 1.** Histopathological appearance of the four major cell types of ovarian sex cord-stromal tumors. A, adult-type GCT, positive for the FOXL2 mutation; B, thecoma; C, steroid cell tumor; and D, Sertoli/Leydig cell tumor, hematoxylin-eosin, 200×. E, FOXL2 protein domain structure of the forkhead sequence (black letters, the surrounding sequence is grayed) showing the site of the mutation (arrow).
to be answered with respect to FOXL2 protein conformation, interaction partners, and signaling pathways.

In summary, the findings provide a clear indication of how massively parallel sequencing will change our understanding of cancer. As the FOXL2 402C→G mutation occurs in almost all A-GCTs but not in other tumors, it could potentially be used as a diagnostic test for this cancer. In addition, further research into the signaling implication of this mutation could lead to the development of targeted therapies for women with advanced or recurrent disease. Although FOXL2 as a transcription factor does not represent a perfect pharmacological target, further insights into its function and downstream effects may identify targetable molecular alterations in these tumors.

Disclosure of Potential Conflicts of Interest

British Columbia Cancer Agency: provisional patent filed on the FOXL2 mutation in A-GCTs.

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