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Original contribution

An immunohistochemical and molecular analysis of problematic and unclassified ovarian sex cord–stromal tumors☆

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Summary Most ovarian sex cord–stromal tumors (SCSTs) can be categorized on the basis of conventional histology, but approximately 10% of cases are unclassified because they present indeterminate or overlapping morphologic features. Immunohistochemical and molecular studies of unclassified ovarian SCST are very limited, but recently, it has been demonstrated that 2 major subgroups of SCST, adult-type granulosa cell tumor and Sertoli-Leydig cell tumor, are characterized by somatic mutations in *FOXL2* and *DICER1*, respectively. In this study, 12 diagnostically problematic ovarian SCST, including 9 unclassified tumors, were investigated for *FOXL2* and *DICER1* mutations and for immunohistochemical expression of calretinin, CD56, CD99, estrogen receptor α, estrogen receptor β, FOXL2, inhibin, progesterone receptor, and steroidogenic factor-1. Four of 11 tumors with satisfactory analysis showed a *FOXL2* mutation; 3 of these cases were reported initially as unclassified SCST and 1 as Sertoli-Leydig cell tumor. Conversely, 3 cases with an original diagnosis of granulosa cell tumor were *FOXL2* mutation–negative, and none of 7 tumors with satisfactory analysis demonstrated a *DICER1* mutation. All tumors expressed at least 4 of the immunomarkers examined, although staining was often focal and there was no consistent correlation with tumor morphology. In conclusion, molecular analysis is useful in the assessment of diagnostically challenging ovarian SCST. The absence of *FOXL2* and *DICER1* mutations in most unclassified SCST suggests that these could represent a distinct tumor subgroup with different molecular pathogenesis. Immunohistochemical profiles overlap with those of better categorized SCST, but staining may be focal or negative emphasizing the requirement for antibody panels in diagnostic assessment.

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1. Introduction

Sex cord–stromal tumors (SCSTs) represent approximately 8% of all primary ovarian neoplasms, but cases that include a sex cord component (granulosa cell or Sertoli cell) are relatively rare [1,2]. Indeed, granulosa cell tumors (of both adult and juvenile types) and Sertoli-Leydig cell tumors (SLTs) together account for less than 5% of all primary ovarian malignancies, and therefore, many pathologists encounter these cases infrequently. SCSTs are also characterized by a broad spectrum of morphologic appearances, and therefore, they continue to present diagnostic difficulties in practice, and earlier reviews have shown that a substantial proportion of cases may have been misclassified [3].

A further problem presented by SCST is that 5% to 10% of cases cannot be classified specifically because they show indeterminate histologic appearances or differentiation patterns that overlap between those typically seen in granulosa cell tumors and SLT [1,2]. Seidman [4] presented 32 unclassified SCST from the referral files of the Armed Forces Institute of Pathology and divided the cases into 2 broad histologic categories, namely, those tumors that predominantly showed an undifferentiated spindle cell appearance resembling primitive gonadal stroma (18 cases) and tumors that exhibited more distinct sex cord–like features (14 cases). The overall prognosis was favorable with recurrence in only 2 of the 17 cases, with available follow-up suggesting that unclassified SCSTs have a similar clinical behavior to typical granulosa or Sertoli-Leydig cell neoplasms and do not represent a higher-grade or more aggressive tumor category. Simpson and colleagues [5] reached similar conclusions after a review of 8 unclassified SCST because only 1 of the 7 tumors with follow-up data proved to be clinically malignant. These authors also performed a limited immunohistochemical analysis on their cases using antibodies to cytokeratin, vimentin, and epithelial membrane antigen [5].

There have been significant recent developments in the pathogenesis of SCST, with the recognition that most adult-type granulosa cell tumors (AGCTs) show a specific somatic mutation in *FOXL2*, C134W [6-8], whereas approximately 60% of SLTs show mutations in *DICER1* [9]. Furthermore, patients with germline mutations in *DICER1* may develop SLT together with pleuropulmonary blastoma, multinodular goiter, and Wilms tumor [10,11]. These findings have important implications including the recognition of possible hereditary cancer syndromes in patients with SLT and the potential development of specific molecular-based therapies in SCST generally. It also seems possible that molecular analysis could be useful in the assessment of ovarian tumors that are difficult to classify on routine histopathology. This is supported by a recent study by Kommoss and colleagues [12], who identified *FOXL2* mutations and hence confirmed the diagnosis of AGCT in 6 of 20 diagnostically problematic ovarian tumors. We have explored this possibility further in a series of 12 SCSTs, many of which created diagnostic problems and divergent opinions at initial assessment and 9 of which were ultimately unclassified. Because there are limited data on the immunohistochemical profile of these tumors, the cases were also investigated using a broad panel of antibodies currently used in the diagnosis of ovarian SCST [13].

2. Materials and methods

2.1. Case selection

The study group comprised 12 ovarian SCSTs in which the initial or final diagnosis was unclassified SCST or in which the tumor subtype was revised after a histologic review. The clinical data and the initial and final diagnoses in each case are summarized in Table 1. Eleven tumors were identified during recent population-based reviews of ovarian SCST (excluding pure stromal tumors) presenting in Western Australia [14,15], where they represented 9.3% of all cases accessioned between 1992 and 2012. In comparison, 96 AGCTs, 5 juvenile-type granulosa cell tumors, and 6 SLTs were encountered in the same period. One additional case (case 8, Table 1) initially presented in 1981 but was reviewed after the development of SLT in 2 relatives during the study period. The initial diagnosis in this case was granulosa cell tumor (not otherwise specified), but upon expert review, the diagnosis was revised to SLT. Three additional tumors (cases 4, 10, and 11) were reviewed by external expert gynecologic pathologists. Follow-up data were obtained from review of case records and from the Western Australian Cancer Registry. The study received institutional ethics committee approval.

2.2. Immunohistochemistry

One representative block from each case was selected for immunohistochemistry using the following panel of antibodies: calretinin, CD56, CD99, estrogen receptor (ER) α, ERβ, FOXL2, inhibin α-subunit (hereafter inhibin), progesterone receptor (PR), and steroidogenic factor-1 (SF-1). The antibody sources and dilutions are summarized in Supplementary Table S1. Staining with each antibody was assessed semiquantitatively according to the extent and intensity of staining. Strong staining was equivalent to that seen in appropriate positive control sections for each antibody. The extent and intensity of immunoreactivity generally correlated, and therefore, the tumors were assessed as score − (no staining), score + (weak or moderate staining in <50% of cells), or score ++ (moderate or strong staining in >50% cells). However, variations in staining patterns between morphologically different tumor areas were noted if applicable.

2.3. Molecular analysis

The RecoverAll Total Nucleic Isolation Kit by Ambion (Life Technologies, Grand Island, NY) was used to extract