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Original Article

Novel immunohistochemical marker in the differential diagnosis of sex cord-stromal tumors: SF-1

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Abstract

Aim: Sex cord-stromal tumors are relatively uncommon tumors which constitute approximately 8% of all primary ovarian neoplasms. Morphologic differentiation of non-SCST from SCSTs can be challenging due to microscopic overlap. Immunohistochemistry is beneficial in challenging cases. Inhibin and calretinin have limited sensitivity and specificity, a more sensitive marker is required. SF-1 is known as a promising immunohistochemical marker in the differentiation of SCST from non-SCST ovarian tumors. For this purpose, various non-SCSTs (metastatic and non-metastatic) having morphologic overlap with SCSTs, and multiple SCSTs were stained with SF-1 antibody to elucidate its importance in morphologically challenging cases.

Materials and Methods: Twenty-three SCST, 40 non-SCSTs, and an ectopic adrenal tissue were stained with SF-1, and also the percentage and the intensity were scored. SF-1 immunoreactivity was seen in all 23 SCST with varying degrees of intensity and percentage. In contrast, non-SCSTs were negative in all
regarding to SF-1. Ectopic adrenal gland tissue and ovarian stroma are positive as non-tumoral lesions.

Results: In our series, SF-1 immunoreactivity was seen in all 23 SCST and ectopic adrenal tissue with varying degrees of intensity and diffuseness. In contrast, non-SCSTs were all negative concerning SF-1. In addition; we observed nuclear positivity with SF-1 in 15-75% of the sclerosing stromal tumor cells, whereas inhibin and calretinin were negative in all 4 cases.

Conclusion: Our data shows that SF-1 is a nuclear, reliable and surrogate marker for all SCSTs, and can be used routinely.

Keywords: Immunohistochemistry; differential diagnosis; NR5A1/SF-1; sex cord-stromal tumors

INTRODUCTION

Sex cord-stromal (SCS) tumors constitute approximately 8% of all primary ovarian neoplasms (1,2). Most of them are fibromas and granulosa cell tumors account for %1 of all ovarian tumors and they are commonly adult-type and are frequently seen in postmenopausal women (3, 4). According to 2020 World Health Organization (WHO) classification; ovarian SCS are classified into three groups; pure stromal tumors, (fibroma, cellular fibroma, thecoma, sclerosing stromal tumor etc.), pure sex cord tumors (adult granulosa cell tumor, juvenile granulosa cell tumor, Sertoli cell tumor, etc.), and mixed-sex cord-stromal tumors (Sertoli-Leydig cell tumors, sex cord-stromal tumors, not otherwise specified and gynandroblastoma). Morphologic differentiation of non-SCST from SCS tumors can be challenging due to microscopic overlap. Immunohistochemistry is beneficial in challenging cases. In particular, an inhibin / calretinin / FOXL2 (+) EMA / CK 7 (-) panel is helpful to exclude tumors of epithelial origin (1). Approximately 10% of cases remain unclassified (2). Inhibin has 71% sensitivity and 99% specificity, while calretinin has 97% sensitivity and 85% specificity (5). Moreover, optimal results are not always achieved. Consequently, a more sensitive marker is required. Steroidogenic Factor-1 (SF-1) is a nuclear receptor that is encoded in the NR5A1 gene. It has been identified as the key regulator for gonadal and adrenal functions. Although its role in sex cord differentiation has not documented yet, SF-1 is known as a promising immunohistochemical marker in the differentiation of SCST from non-SCST ovarian tumors (2,3). For this purpose, various non-SCSTs (metastatic and nonmetastatic) having morphologic overlap with SCSTs, and multiple SCSTs were stained with SF-1 antibody to elucidate its importance in morphologically challenging cases.

MATERIALS and METHODS

Ninety SCSTs and 64 non-SCSTs were identified by the Hacettepe University Department of Pathology registry

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system between 2004 and 2016. Using World Health Organization criteria, those cases which paraffin blocks and slides found in the archives were re-evaluated, and the diagnosis were confirmed by an expert pathologist (AU). Cases that did not display a histological differential with SCSTs and consultation cases without paraffin blocks were excluded from the study. Finally, 23 SCST, 40 non-SCST, and an ectopic adrenal tissue were retrieved. Definite diagnosis of 23 SCSTs indicated granulosa cell tumors in 3 cases, Sertoli-Leydig cell tumors in 5 cases, steroid cell tumors in 3 cases, sclerosing stromal tumors in 4 cases, juvenile granulosa cell tumors in 3 cases, a high grade SertoliLeydig cell tumor in 1 case, an undifferentiated sex cord-stromal tumor in 1 case, pregnancy luteoma in 1 case, stromal luteoma in 1 case, and thecoma with minor sex cord-stromal elements in 1 case. Of 40 non-SCST tumors, 12 were metastatic (5 lobular carcinoma metastasis, 6 ductal carcinoma metastasis, 1 metastasis of ductal and lobular carcinoma) while 28 were primary (11 Brenner tumors, 8 endometrial stromal sarcoma, 7 mesothelioma, 1 borderline Brenner tumor, 1 hypercalcemic type small cell carcinoma). 5 microne sections were stained using an automated Leica Bond Max stainer (Shandon, Frankfurt, Germany). After heat-induced antigen retrieval (ETDA based), a primary antibody (SF-1 Clone N1665, Mouse Monoclonal Antibody, Ready-to-use, Thermo Scientific, USA) was applied and incubated for 30 minutes. Antigen detection was performed according to the recommended procedure. Diaminobenzidine was used as the chromogen, and counterstained with hematoxylin. Evaluation of the slides was performed with internal and external controls. Any degree of nuclear staining was accepted as positive. For positively staining tumors, diffuseness scoring was performed based on the percentage of the nuclear positive cells. A score of 0 indicated fewer than 10% positive tumor cells, a score of 1 indicated that positive staining was present in 10-49% of the tumor cells, a score of 2 indicated 50-75% positive reaction of the neoplastic cells, a score 3 indicated that positive staining was present in >75% of the tumor. The degree of intensity was scored 0 in the absence of any staining, weak staining is scored as 1, moderate staining was scored as 2 and strong staining was scored as 3. This study was conducted with the approval of the Ethics Committee of Non-Interventional Clinical Applications of Hacettepe University (approval number 16969557-387).

RESULTS

SF-1 immunoreactivity was seen in all 23 SCST and ectopic adrenal tissue with varying degrees of intensity and diffuseness. In contrast, non-SCSTs were all negative concerning SF-1. 5 Sertoli-Leydig cell tumors (Figure 1, 2), 3 steroid cell tumors, 3 juvenile granulosa cell tumors, 1 undifferentiated sex cord-stromal tumor, 1 pregnancy luteoma and 1 thecoma with minor sex cord-stromal elements showed strong staining in 75% or more of the cells. 4 cases (3 granulosa cell tumors and 1 stromal luteoma) showed strong staining in 50-75% of the cells, 5 cases (4 sclerosing stromal tumors (Figure 3, 4 and Table 1) and 1 high-grade Sertoli-Leydig cell tumor) showed variable positive staining. We searched the archive for the immunohistochemical stains of inhibin and calretinin for four sclerosing stromal tumors in our series and inhibin and calretinin were found to be negative in all four cases. In the case of 40 non-SCSTs (lobular carcinoma and ductal carcinoma of breast, benign and borderline Brenner tumors, endometrial stromal sarcoma, mesothelioma, hypercalcemic type small cell carcinoma) there was complete negativity concerning SF-1 except in the case of ectopic adrenal. Ectopic adrenal tissue reacted strongly in 50-75% of the cells. Besides, in 10 cases, tumor blocks include normal ovarian parenchyma; all of which were shown to be weakly and diffusely nuclear positive (Figure 5).

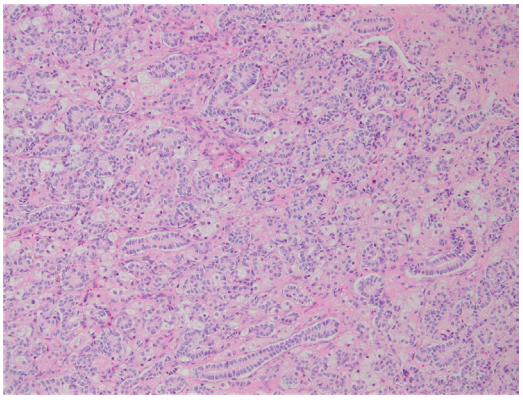

Figure 1. Sertoli-Leydig cell tumor, H&E (x100)

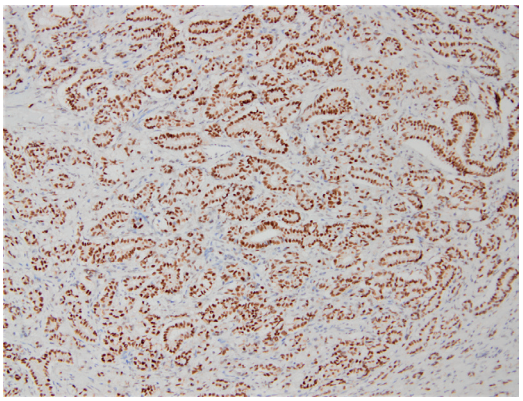

Figure 2. Sertoli-Leydig cell tumor, more than 75% of tumor cells are strongly nuclear positive with SF-1 (x100)

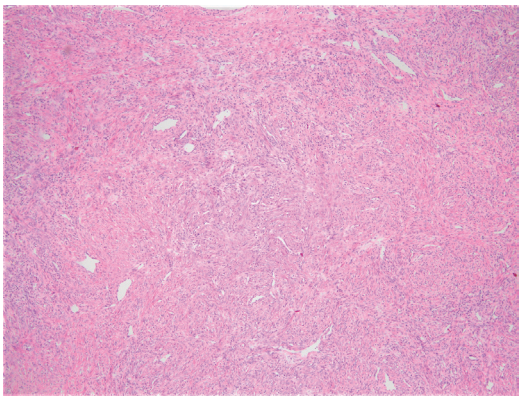

Figure 3. Sclerosing stromal tumor, H&E (x100)

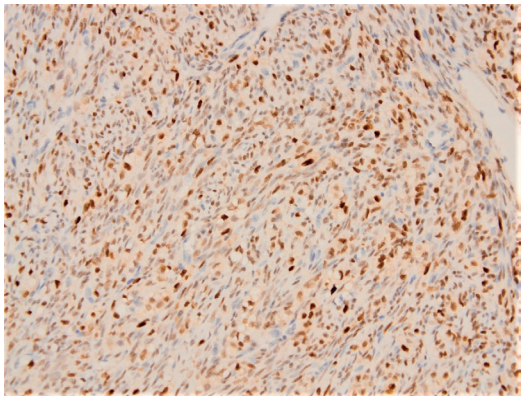

Figure 4. Sclerosing stromal tumor, 50-75% tumor cells are positive with SF-1 (x100)

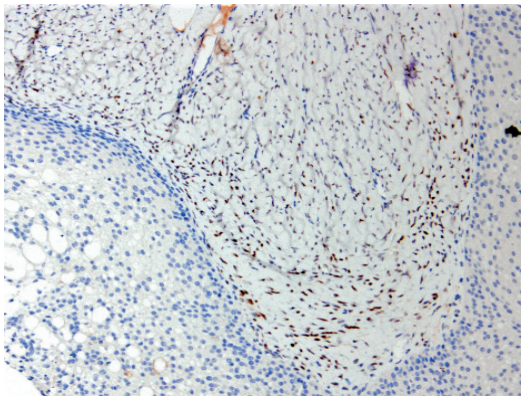

Figure 5. Metastatic breast carcinoma, all tumor cells are negative while ovarian stroma is positive with SF-1 (x100)

Table 1. Staining percentage and intensity scoring of SF-1 of tumor cells in sclerosing stromal tumors

Case number of sclerosing stromal tumors	SF-1 Staining (%) and Score	SF-1 Staining Intensity Score
1	>75% (Score 3)	Strong staining (Score 3)
2	>75 % (Score 3)	Strong staining (Score 3)
3	30% (Score 1)	Weak staining (Score 1)
4	15% (Score 1)	Moderate staining (Score 2)

DISCUSSION

Ovarian masses are usually asymptomatic and diagnosed incidentally by ultrasonographic examination. They are most often symptomatic when complications exist as torsion, hemorrhage or rupture, and the most common symptom is abdominal pain (6,7). Sex cord-stromal tumors (SCST) account for approximately 8% of primary ovarian neoplasms (1). These tumors comprise a heterogeneous group and are formed by diverse cell types that arise from the primitive sex cords or stromal cells. The stromal cells include theca cells, fibroblasts, and Leydig cells whereas the gonadal primitive sex cords include granulosa cells and Sertoli cells (8-10). Such tumors have a wide spectrum of morphological appearances (2). Sometimes, due to morphological overlap and diversity, SCST can be confused with non-SCST, especially the ones metastasized to the ovary. For this reason, immunohistochemistry is a frequently applied method for differential diagnosis. Even though it has low sensitivity, the hormone polypeptide inhibin has been used for many years as a conventional marker for SCST (4,5,11). Also, a mesothelium marker – calretinin - which is more sensitive than the aforementioned marker for ovarian SCST, has also been widely used (5). Recently, inhibin and calretinin have been compared, and it has been suggested that other markers such as EMA, should be added to the panel (12). In addition to conventional markers, CD56, WT-1, CD99, and FOXL2 have also been found to have an important role to play in SCST diagnosis (11,13-15). Lately, FOXL2 and DICER1 mutations have been identified as crucial diagnostic tools, and offer promising therapeutic options (1,2,16-19). SF-1 is a nuclear transcription factor that regulates the steroidogenesis gene for gonads and adrenal gland development, sexual differentiation, reproduction, and metabolism. It is reported to be a sensitive and specific marker for Sertoli cell tumors and is diagnostically comparable with other conventional sex cord-stromal markers (3). By obtaining strong nuclear positivity in Sertoli and Leydig cell tumors, Sangoi AR. et al. found supportive evidence that SF-1 was not expressed in testicular germ cell tumors and was a specific marker for SCST (20). By obtaining 100% positive staining in Sertoli cell tumors, Zhao et al. compared WT-1 and inhibin in the differential diagnosis of ovarian Sertoli cell tumors and other tumors and found that SF-1 is a useful marker (3). In a study by Irving et al. of microcystic stromal tumors (MST), SF-1, FOXL2, and WT-1 showed diffuse nuclear positivity and SF-1 showed positivity in the differential diagnosis of ovarian SCSTs and supported the diagnosis of MST (21). Sclerosing stromal tumors, a rare SCST, often presents with diagnostic difficulty as its immunoreactivity is variable. Uterine tumor resembling sex cord stromal tumors (UTROSCT) are also difficult area of gynecologic diagnostic pathology, especially in differentiating from its potential histologic mimics. Recently it was shown that SF-1 is %100 specific in UTROSCT (22). A distinct entity called female adnexal tumor (FATWO) is a rare tumor resembles endometrioid adenocarcinoma and Sertoli-Leydig cell tumors. PAX8 and SF-1 immunohistochemistry was found to be helpful marker in the distinction between FATWOs and tumors with FATWO-like areas that is, endometrioid adenocarcinomas with a prominent spindle cell component and SCTs, respectively (23). Moreover, SF-1 and SALL-4 are sensitive and specific markers could be used to distinguish yolk sac tumor and sex cord stromal tumors in difficult situations (24). Our study includes SCSTs and morphologically SCST mimicking non-SCSTs. Our data could be helpful in routine practice in that it gives exact data for the reliability of SF-1 in morphologically SCST mimicker non-SCST cases. Moreover, our series includes 4 sclerosing stromal tumors; all of which is positive whereas, inhibin and calretinin were negative in

all 4. Two sclerosing stromal tumor is positive with more than %75 of the tumor cells with score 3 intensity, 1 case has positive staining of %30 tumor cells with score 1 intensity, 1 sclerosing stromal cell tumor is positive with %15 distribution with score 3 intensity (Table 1). Therefore, positive staining with SF-1 is an important finding in the case of sclerosing stromal tumors. It seems that this is the first data concerning SF-1 expression in sclerosing stromal tumors in the English literature. Additionally, SF-1 is consistently negative in morphologically mimicker non-SCSTs. Ectopic adrenal tissue and ovarian stroma is SF-1 positive non-tumoral lesions. Zhao et al. also showed a positive ovarian stromal reaction with SF-1 in their sertoli cell tumor series (3). The SF-1 interpretation facility should be noted as a nuclear marker. As a conclusion, in this paper, it is reported that nuclear SF-1 is positive in all SCST cases, including sclerosing stromal tumors, and negative for all the non-SCST cases, regardless of tumor origin (primary or metastatic).

CONCLUSION

Thus, our data shows that SF-1 is a nuclear, reliable and surrogate marker for all SCSTs, and can be used in routine diagnostic practice.

Competing Interests: The authors declare that they have no competing interest.

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Ethical Approval: All procedures in this study were approved by the Ethics Committee of Non-Interventional Clinical Applications of Hacettepe University. The approval number is 16969557-387.

REFERENCES

1. Al-Agha OM, Huwait HF, Chow C, et al. FOXL2 is a sensitive and specific marker for sex cord-stromal tumors of the ovary. Am J Surg Pathol 2011;35:484-94.
2. Stewart CJ, Alexiadis M, Crook ML, Fuller PJ. An immunohistochemical and molecular analysis of problematic and unclassified ovarian sex cord-stromal tumors. Hum Pathol 2013;44:2774-81.
3. Zhao C, Barner R, Vinh TN, et al. SF-1 is a diagnostically useful immunohistochemical marker and comparable to other sex cord-stromal tumor markers for the differential diagnosis of ovarian sertoli cell tumor. Int J Gynecol Pathol 2008;27:507-14.
4. Rishi M, Howard LN, Bratthauer GL, Tavassoli FA. Use of monoclonal antibody against human inhibin as a marker for sex cord-stromal tumors of the ovary. Am J Surg Pathol 1997;21:583-9.
5. Movahedi-Lankarani S, Kurman RJ. Calretinin, a more sensitive but less specific marker than alpha-inhibin for ovarian sex cord-stromal neoplasms: an immunohistochemical study of 215 cases. Am J Surg Pathol 2002;26:1477-83.
6. Yalcin Comert HS, Eyuboglu I, Ersoz S, Aet al. Ovarian masses in children: A single center's experience. Ann Med Res 2020;27:1465-8.
7. Cevik M, Boleken ME. Characteristics and Approach of Ovarian Masses in Children: Series of 34 Cases. Van Med J 2013;20:57-61.
8. Haroon S, Zia A, Idrees R, et al. Clinicopathological spectrum of ovarian sex cord-stromal tumors; 20 years' retrospective study in a developing country. J Ovarian Res 2013;6:87.
9. Shim SH, Kim DY, Lee SW, et al. Laparoscopic management of early-stage malignant non-epithelial ovarian tumors: surgical and survival outcomes. Int J Gynecol Cancer 2013; 23:249-55.
10. Jung SE, Rha SE, Lee JM, et al. CT and MRI findings of sex cord-stromal tumor of the ovary. Am J Roentgenol 2005;85:207-15.
11. Rathore R, Arora D, Agarwal S, Sharma S. Correlation of FOXL2 with Inhibin and Calretinin in the Diagnosis of Ovarian Sex Cord Stromal Tumors. Turkish J of Pathology 2016;33:121-8.
12. Deavers MT, Malpica A, Liu J, et al. Ovarian sex cord-stromal tumors: an immunohistochemical study including a comparison of calretinin and inhibin. Mod Pathol 2003;16:584-90.
13. McCluggage WG, McKenna M, McBride HA. CD56 is a sensitive and diagnostically useful immunohistochemical marker of ovarian sex cord-stromal tumors. Int J Gynecol Pathol 2007;26:322-7.
14. Zhao C, Bratthauer GL, Barner R, Vang R. Diagnostic utility of WT1 immunostaining in ovarian sertoli cell tumor. Am J Surg Pathol 2007;31:1378-86.
15. Zhao C, Vinh TN, McManus K, 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;33:354-66.
16. D'Angelo E, Mozos A, Nakayama D, Espinosa I, Catasus L, Munoz J et al. Prognostic significance of FOXL2 mutation and mRNA expression in adult and juvenile granulosa cell tumors of the ovary. Mod Pathol 2011;24:1360-7.
17. Georges A, Auguste A, Bessiere L, Vanet A, Todeschini AL, Veitia RA. FOXL2: a central transcription factor of the ovary. J Mol Endocrinol 2013;52:R17-33.
18. Goulvent T, Ray-Coquard I, Borel S, Haddad V, Devouassoux-Shisheboran M, Vacher-Lavenu MC et al. DICER1 and FOXL2 mutations in ovarian sex cord-stromal tumours: a GINECO Group study. Histopathology 2016;68:279-85.
19. Kommoss S, Gilks CB, Penzel R, Herpel E, Mackenzie R, Huntsman D, et al. A current perspective on the pathological assessment of FOXL2 in adult-type granulosa cell tumours of the ovary. Histopathology 2014;64:380-8.
20. Sangoi AR, McKenney JK, Brooks JD, Higgins JP. Evaluation of SF-1 expression in testicular germ cell tumors: a tissue microarray study of 127 cases. Appl Immunohistochem Mol Morphol 2013;21:318-21.
21. Irving JA, Lee CH, Yip S, Oliva E, McCluggage WG, Young RH. Microcystic Stromal Tumor: A Distinctive Ovarian Sex Cord-Stromal Neoplasm Characterized by FOXL2, SF-1, WT-1, Cyclin D1, and beta-catenin Nuclear Expression and CTNNB1 Mutations. Am J Surg Pathol 2015;39:1420-6.

22. Stewart CJ, Crook M, Tan A. SF1 immunohistochemistry is useful in differentiating uterine tumours resembling sex cord-stromal tumours from potential histological mimics. Pathology 2016;48:434-40.
23. Goyal A, Masand RP, Roma AA . Value of PAX-8 and SF-1 Immunohistochemistry in the Distinction Between Female Adnexal Tumor of Probable Wolffian Origin and its Mimics. Int J Gynecol Pathol 2016;35:167-75.
24. Bai S, Wei S, Ziober A, Yao Y, Bing Z (2013) SALL4 and SF-1 are sensitive and specific markers for distinguishing granulosa cell tumors from yolk sac tumors. Int J Surg Pathol 2013;21:121-5.