Evaluation of SF-1 Expression in Testicular Germ Cell Tumors: A Tissue Microarray Study of 127 Cases

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Abstract: Differentiating testicular germ cell tumors from sex-cord stromal tumors can be difficult in certain cases because of overlapping morphologic features and/or an absence of clinically apparent hormonal symptoms. Immunohistochemistry may be needed as an ancillary diagnostic tool in this differential diagnostic setting. Steroidogenic factor-1 (SF-1) is a nuclear transcription factor controlling steroidogenesis and is expressed in developing Sertoli and Leydig cells. Although 1 recent study has reported SF-1 nuclear immunoreactivity in testicular sex-cord stromal tumors, the specificity for this marker in germ cell tumors has not been evaluated. After encountering several problematic cases (including some on testicular biopsy), we sought to determine the diagnostic specificity of SF-1 in a large series of germ cell tumors. Nuclear immunohistochemical expression of SF-1 was evaluated in 127 germ cell tumors using tissue microarray technology with 23 non–germ cell tumor tissues as positive internal controls. No nuclear SF-1 expression was identified in any of the 127 germ cell tumors [including choriocarcinoma (3), embryonal carcinoma (25), epidermal inclusion cyst (1), intratubular germ cell neoplasia unclassified (4), seminoma (72), spermatocytic seminoma (2), teratoma (8), and yolk sac tumor (12)]. All 23 non–germ cell tumor tissues showed strong nuclear SF-1 expression in Sertoli and/or Leydig cells [including testicular atrophy (10), cryptorchidism (2), normal testis (4), hypospermatogenesis (1), immature testis (1), intratubular large cell calcifying Sertoli cell tumor (1), Leydig cell tumor (3), and Sertoli only (1)]. This study documents the absence of SF-1 expression in testicular germ cell tumors and supports its specificity for sex-cord stromal lesions in this diagnostic context.

Key Words: germ cell tumor, immunohistochemistry, SF-1, sex-cord stromal, Sertoli, Leydig

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The distinction between testicular germ cell tumors and sex-cord stromal tumors can generally be accomplished by both clinical and morphologic features. However, in certain cases an absence of hormonal symptoms compounded with overlapping histologic growth patterns and a lack of identifiable intratubular germ cell neoplasia can make this distinction difficult. In these situations, immunohistochemistry may be a useful diagnostic tool. The diagnostic utility of image-guided needle biopsies for small testicular lesions has been suggested[1]; however, we have encountered several biopsies that contained only scant tissue with biopsy-induced artifact that further complicated this distinction in which immunohistochemistry was key in arriving at an accurate diagnosis.

Although the transcription factor SALL4 has been extensively studied as a specific nuclear marker for testicular germ cell tumors in the diagnostic distinction from sex-cord stromal tumors,[2] Steroidogenic factor-1 (SF-1) (a nuclear marker with a proposed similar specificity for testicular sex-cord stromal tumors) has not been fully evaluated. Therefore, in this study we investigate the diagnostic specificity of SF-1, a nuclear transcription factor controlling gonadal steroidogenesis, in a large series of testicular germ cell tumors.

MATERIALS AND METHODS

A tissue microarray (TMA) composed of 100 randomly distributed germ cell tumors using 1.2-mm diameter cores was prepared in triplicate (Stanford TMA 136) and evaluated as described elsewhere.[3] The 100 testicular tumors represented in the TMA included: 1 choriocarcinoma, 21 embryonal carcinomas, 2 intratubular germ cell neoplasia unclassified, 62 seminomas, 1 spermatocytic seminoma, 5 teratomas, and 8 yolk sac tumors. An additional TMA containing 27 germ cell tumors using 3-mm diameter cores was also prepared in duplicate (El Camino TMA 3) and similarly evaluated. The 27 testicular tumors represented in this TMA included: 2 choriocarcinomas, 1 epidermal inclusion cyst, 4 embryonal carcinomas, 2 intratubular germ cell neoplasia unclassified, 10 seminomas, 1 spermatocytic seminoma, 3 teratomas, and 4 yolk sac tumors. Immunohistochemical expression of SF-1 (1:100, clone N1665; R&D Systems, Minneapolis, MN) was evaluated using the standard avidin-biotin technique with a Dako (Carpinteria, CA) autostainer with citrate retrieval on 4-mm-thick formalin-fixed, paraffin-embedded freshly

cut sections mounted on charged slides and baked at 60°C for 1 hour. Seventeen non–germ cell tumor tissues [including atrophic testis (8), cryptorchidism (2), normal testis (4), hypospermatogenesis (1), immature testis (1), and Leydig cell tumor (1)] also present on the larger TMA were used as positive internal controls. Six non–germ cell tumor

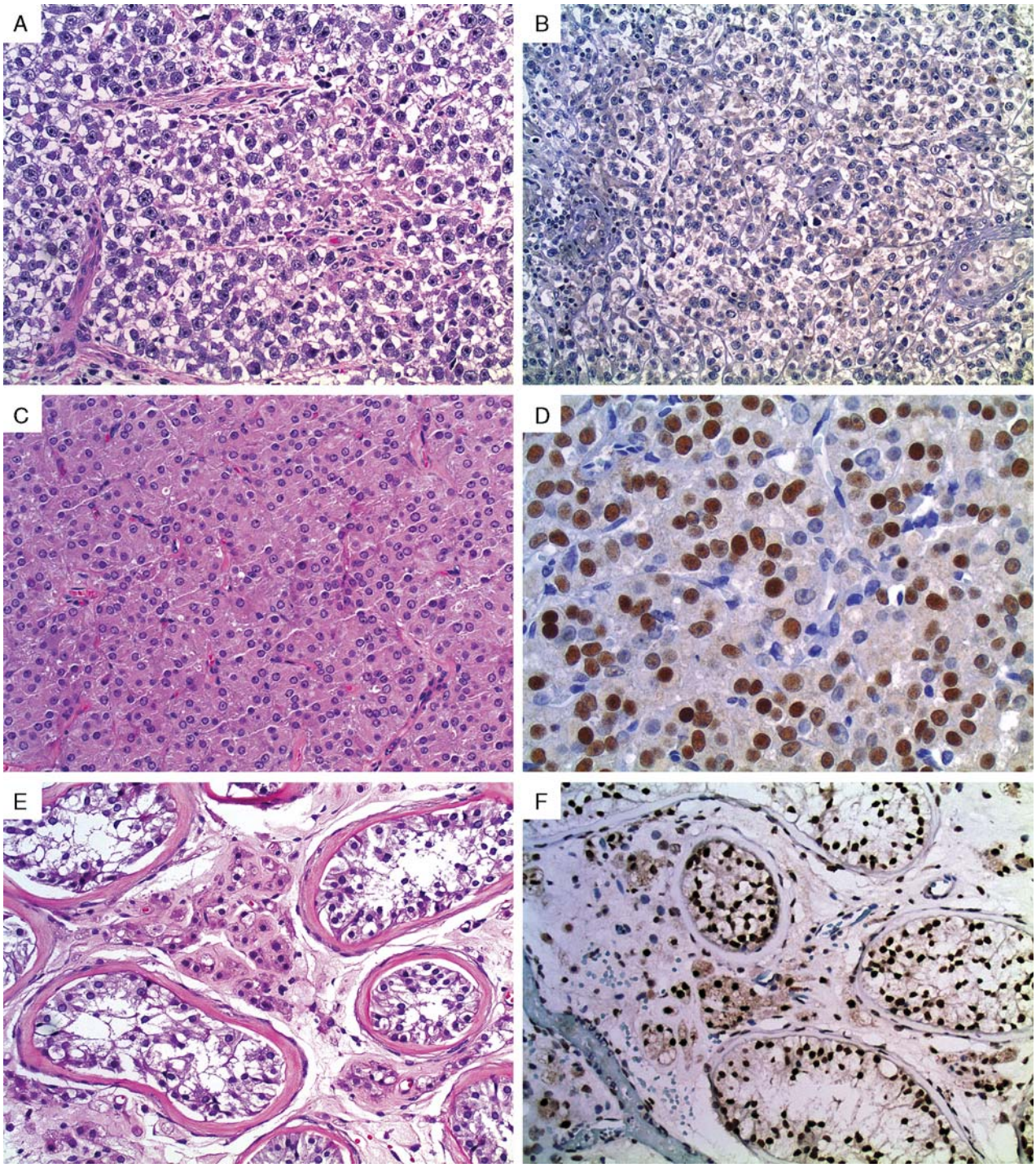

FIGURE 1. A and B, Negative expression for SF-1 in seminoma (original magnification, ×200). C and D, Diffuse strong nuclear expression for SF-1 in Leydig cell tumor (original magnification, ×100, ×400). E, Germ cell aplasia (Sertoli-only syndrome; original magnification, ×200). F, SF-1 shows strong nuclear expression within Sertoli cells lining hyalinized seminiferous tubules as well as within intertubular Leydig cells (original magnification, ×200). (A, C, and E are hematoxylin and eosin stains; B, D, and F are immunostains).

tissues [including atrophic testes (2), intratubular large cell calcifying Sertoli cell tumor (1), Leydig cell tumors (2), and Sertoli only (1)] also present on the smaller TMA were used as positive internal controls. Separate positive and negative external controls (adrenal cortex and normal colon, respectively) were also utilized. Only nuclear immunoreactivity was manually scored as positive with staining intensity scored as none (0), weak (1 to 2+/4+), or strong (3 to 4+/4+) by 2 authors (A.R.S. and J.K.M.) with any potential disagreement in scoring assessed by a third author (J.P.H.).

RESULTS

All 127 germ cell tumors were evaluable after immunostaining and yielded consistent scoring results by both authors. There was no nuclear expression for SF-1 identified in any of the 127 germ cell tumors (Fig. 1, Table 1). Ten cases of seminoma showed strong intratumoral positive control staining within entrapped Leydig cells. All 23 non–germ cell tumor tissues showed strong, diffuse SF-1 nuclear immunoreactivity in Sertoli and/or Leydig cells (Fig. 1, Table 2).

DISCUSSION

Among the many diagnostically challenging areas in testicular tumor histopathology, separating sex-cord stromal tumors from germ cell tumors remains one of the most clinically relevant distinctions given the extraordinary sensitivity of germ cell tumors to modern treatment regimens. A clinical history of hormonal symptoms may be useful in alerting the pathologist to the possibility of a sex-cord stromal tumor; however, that information is commonly absent or unknown. There are multiple series describing morphologic mimicry in this differential diagnostic distinction including the diffuse pattern of malignant Sertoli cell tumors with a clear cytoplasm, prominent nucleoli, and lymphocytic inflammation (mimicking seminoma),[4,5] Leydig cell tumors with prominent cysts (mimicking yolk sac tumor),[6] seminomas with marked tubule formation (mimicking sex-cord stromal tumors),[7–9] and sex-cord stromal tumors with entrapped germ cells (mimicking true mixed germ cell–sex-cord stromal tumors).[10] Moreover, although a recent study describes macroscopic Sertoli cell nodules presenting as mass lesions with a differential diagnosis that includes Sertoli cell tumors,[11] we recently encountered such a situation on a testicular mass biopsy in which the differential also included a germ cell tumor (in particular, seminoma).

TABLE 1. Immunostaining Results of SF-1 Antibody in Germ Cell Tumors

Germ Cell Tumor	Positive Cases
Choriocarcinoma	0/3
Embryonal carcinoma	0/25
Epidermal inclusion cyst	0/1
Intratubular germ cell neoplasia	0/4
Seminoma	0/72
Spermatocytic seminoma	0/2
Teratoma	0/8
Yolk sac tumor	0/12
Total	0/127

TABLE 2. Immunostaining Results of SF-1 Antibody in Non–Germ Cell Tumor Tissues

Non–Germ Cell Tumor Tissue	Positive Cases*
Atrophic testis	10/10
Cryptorchidism	2/2
Normal testis	4/4
Hypospermatogenesis	1/1
Immature testis	1/1
Intratubular large cell calcifying Sertoli cell tumor	1/1
Leydig cell tumor	3/3
Sertoli only	1/1
Total	23/23

*Reactivity in Sertoli and/or Leydig cells.

These examples of morphologic mimicry are scenarios in which immunohistochemistry may be a useful ancillary diagnostic tool. At present, SALL4 and inhibin are the commonly recommended immunohistochemical markers to separate sex-cord stromal tumors from germ cell tumors, respectively.[2,12–15] However, in our experience inhibin shows variable sensitivity among the various sex-cord stromal tumors with notably poorer sensitivity in Sertoli cell tumors, a finding noted by others.[14] More importantly, we have previously described problematic issues of interpreting "positive" results with inhibin in other steroid-producing tumors depending on one's cutoff for cytoplasmic staining intensity,[16] an issue typically obviated by a nuclear antibody.

SF-1 (also known as adrenal-4 binding protein) is well recognized in the developmental pathology literature as a nuclear transcription factor regulating steroidogenesis in the gonads and adrenal gland with expression identified in the testis, ovary, adrenal gland, pituitary gland, and placenta.[17,18] More recent investigation of SF-1 expression has focused on neoplasms of the adrenal cortex and ovarian sex-cord stromal tumors.[16–18] Although some studies[19,20] investigating the immunoreactivity of SF-1 in testicular sex-cord stromal tumors have shown moderate overall diagnostic sensitivity versus the strong sensitivity previously described in their ovarian counterparts,[17,18] to our knowledge, the specificity of SF-1 in testicular germ cell tumors has not been previously studied and led to the current investigation.

In this study, SF-1 did not show immunoreactivity in any of the germ cell tumors in a TMA analysis of 127 unique cases with good internal control staining (Table 1). Although SF-1 showed 100% sensitivity among 23 non–germ cell tumor tissues containing Sertoli and/or Leydig cells (Table 2), admittedly only 4 of these cases were neoplastic, precluding a definitive assessment on incorporating SF-1 as a diagnostically sensitive marker for sex-cord stromal tumors from the current study alone. Although SF-1 is not equally as sensitive among testicular

sex-cord stromal tumors as compared to ovarian sex-cord stromal tumors based on the aforementioned studies,[17–20] SF-1 may prove a useful nuclear sex-cord stromal antibody addition to an immunohistochemical panel of SALL4 and inhibin in the differential diagnosis of sex-cord stromal tumors versus germ cell tumors.

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