Nuclear localization of β-catenin in Sertoli cell tumors and other sex cord–stromal tumors of the testis: an immunohistochemical study of 87 cases

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Abstract

The diagnosis and subclassification of Sertoli cell tumors (SCT) of the testis are often challenging to general surgical pathologists due to the rarity of the tumors. Immunohistochemistry study to date has limited diagnostic value. Nuclear localization of β-catenin, which correlated closely with *CTNNB1* gene mutation, was recently reported in SCTs. We investigated the utility of β-catenin nuclear localization in diagnosing SCTs and differentiating them from other testicular sex cord–stromal tumors. Immunohistochemistry staining for β-catenin was evaluated in 87 cases of testicular sex cord–stromal tumor: 33 SCTs, not otherwise specified (SCT-NOS) (15 with benign and 18 with malignant features), 10 sclerosing Sertoli cell tumors (SSCT), 5 large cell calcifying Sertoli cell tumors (LCCSCT), 6 Sertoli–stromal cell tumors, 10 Leydig cell tumors, 7 juvenile granulosa cell tumors, 4 adult granulosa cell tumors, and 12 sex cord–stromal tumors, unclassified. Twenty-one of 33 (64%) SCT-NOS, 6 of 10 (60%) SSCTs, and 4 of 6 (67%) Sertoli–stromal cell tumors showed strong, diffuse β-catenin nuclear and cytoplasmic staining. Nuclear β-catenin positivity was more frequent in SCTs-NOS with benign features than in those with malignant features (93% and 39%, respectively, $p = 0.13$). Nuclear staining of β-catenin was seen only in the Sertoli components of the Sertoli–stromal cell tumors. All 5 LCCSCTs and all other types of sex cord–stromal tumor were negative for β-catenin nuclear staining. In conclusion, SCT-NOS and SSCT frequently show β-catenin nuclear localization. Positive nuclear staining of β-catenin is specific for SCT-NOS, SSCT, and Sertoli–stromal cell tumor among testicular sex cord–stromal tumors but has limited sensitivity (63%) in this group. The similar reactivity of SCT-NOS and SSCT provides additional support that these two variants are not distinct entities.

Keywords: Sertoli cell tumor; β-catenin; immunohistochemistry; sex cord-stromal tumor; testicular neoplasms.

Introduction

Sertoli cell tumors (SCT) of the testis are most common in middle age and represent approximately 1% of all testicular tumors[1]. Most SCTs are of the "not otherwise specified (NOS)" type, but there are separate variants including the large cell calcifying Sertoli cell tumor (LCCSCT), intratubular large cell hyalinizing Sertoli cell neoplasia, and the sclerosing Sertoli cell tumor (SSCT).[1-5] The former two variants are associated with two important clinical conditions, the Carney complex and Peutz-Jeghers syndrome, respectively, which makes their distinction from SCT-NOS and other sex cord-stromal tumors critical. The differentiation of SCT from other sex cord–stromal tumors and the determination of SCT variants are mainly based on histological examination, which can be challenging to general surgical pathologists due to the rarity of these neoplasms. Immunohistochemistry (IHC) staining for various markers, including inhibin, calretinin, and steroidogenic factor-1, have shown limited diagnostic value in differentiating among SCTs and other tumors in the sex cord-stromal group.[6, 7]

β-catenin is a component of adherens junctions on the cell membranes.[8] Under certain neoplastic conditions, this protein accumulates in the nucleus, and nuclear localization of β-catenin has been reported as a useful diagnostic marker for some tumors such as desmoid tumor, hepatoblastoma, and pancreatic solid pseudopapillary tumor.[9-11] Consistent β-catenin nuclear staining has also been reported in ovarian microcystic stromal tumors.[12, 13] The expression of β-catenin in nonneoplastic Sertoli cells is limited to the cytoplasm and membranes.[14] Recently, Perrone et al reported 14 cases of SCT in which β-catenin nuclear localization was demonstrated in a high proportion of cases (10/14).[15] One case of SSCT with β-catenin nuclear localization was later reported by the same group[16] Furthermore, this reactivity correlated highly with mutation in the *CTNNB1* gene, which encodes for β-catenin and leads to its accumulation.[15, 16] A

case of bilateral SCTs in a patient with familial adenomatous polyposis, a condition associated with germline mutation in the *APC* gene (whose non-mutated protein normally participates in β-catenin degradation), showed strong nuclear β-catenin immunostaining.[17] These findings suggest that β-catenin nuclear localization may play an important role in the formation of some SCTs and also may be used as a potential marker for the diagnosis of SCTs. However, the exact frequency of β-catenin nuclear localization in SCTs of testis is unknown because of the limited number of cases reported to date; and it is furthermore unknown whether nuclear β-catenin staining is specific for SCTs among testicular sex cord–stromal tumors. In this study, we evaluated the status of β-catenin nuclear localization in a relatively large cohort of SCTs, as well as in various other testicular sex cord–stromal tumors.

Materials and Methods

Case selection: A total of 87 resected sex cord–stromal tumors (including 33 SCTs-NOS [15 with benign features and 18 with malignant features, according to the criteria of Young et al[1]], 5 LCCSCTs, 10 SSCTs, 6 Sertoli–stromal cell tumors, 10 Leydig cell tumors, 7 juvenile granulosa cell tumors, 4 adult granulosa cell tumors, and 12 unclassified sex cord–stromal tumors) over the past 20 years were identified from the pathology archive. Most were consultation cases. We reviewed hematoxylin and eosin-stained slides from all cases, with the diagnosis confirmed by the senior author (T.M.U.). All of the malignant SCT cases included in this series had at least two of the previously established criteria for malignancy in SCTs, and/or biologic evidence of malignancy including invasion and metastasis to other organs.[1] None of the benign SCT cases in this study had more than one of the above-mentioned criteria. Institutional review board permission was granted for the use of de-identified tissue samples.

Immunohistochemistry: IHC staining with anti-β-catenin monoclonal antibody (Cell Marque, Rocklin, CA) was performed on a single representative block from each case by using high pH antigen retrieval and Envision Plus DAB detection system (Dako, Carpinteria, CA). Appropriate positive and negative controls were used. The results were scored by two independent observers (C.Z. and T.M.U.) using a semiquantitative graded criteria: Score 0: No staining or weak nonspecific staining; Score 1, moderate to strong nuclear staining in 1–5% of tumor cells; Score 2, moderate to strong nuclear staining in 6–50% of tumor cells; Score 3, moderate to strong nuclear staining in 51–100% of tumor cells. Positive β-catenin staining was defined as Score 1 or above.

Statistics: The frequency of β-catenin nuclear positivity was compared between SCT-NOS groups with benign and malignant features using the Chi square test. A p value of <0.05 was considered significant.

Results

β-catenin nuclear, cytoplasmic and membranous staining patterns were evaluated and scored in all 87 cases of sex cord–stromal tumors and in the surrounding nonneoplastic testicular parenchyma, if present. β-catenin IHC staining results are summarized in Table 1.

Strong nuclear immunoreactivity was seen in 21 of 33 cases of SCT-NOS (14 of 15 cases of benign SCT-NOS, 7 of 18 malignant SCT-NOS) (Figs. 1A-B), and 6 of 10 cases of SSCT (Figs. 1C-D). β-catenin nuclear reactivity among SCT-NOS with benign features was more frequent than in those with malignant features, but the difference was not statistically significant (93% vs. 39%, $p = 0.13$). In the 6 cases of Sertoli-stromal cell tumor, β-catenin nuclear staining was seen only in the Sertoli cell component of 4 (Fig. 1E-F). Among the positive cases, 20 SCTs-NOS (13 benign and 7 malignant), 5 SSCTs and 1 Sertoli-stromal cell tumor had a score

of 3+, 1 benign SCT-NOS, 1 SSCT and 2 Sertoli-stromal cell tumors had a score of 2+, and 1 Sertoli-stromal cell tumor had a score of 1+. No nuclear staining was seen in any of the 5 LCCSCTs, 12 sex cord–stromal tumors, unclassified, 10 Leydig cell tumors, 7 juvenile granulosa cell tumors, and 4 adult granulosa cell tumors. Most cases of all categories (76 of 87) showed positive membranous and cytoplasmic staining (Table 1). The nonneoplastic testicular parenchyma showed membranous reactivity of β-catenin in Sertoli cells and spermatogenic cells.

Discussion

Sex cord–stromal tumors of the testis can be considered in four major groups: Leydig cell tumors, SCTs, granulosa cell tumors, and unclassified. Correct classification of these tumors is important for clinical management; however, most pathologists have limited experience with them because of their rarity. IHC to date has not generally been useful in subclassification, especially in differentiating SCTs from others, because of absence of a specific immunomarker.

Results of our study show frequent (over 60%) positive nuclear staining for β-catenin in SCT-NOS and SSCT, which is in agreement with a previous report by Perrone et al.[15] Additionally, our results suggest that β-catenin nuclear staining is completely specific for SCTs among all sex cord–stromal tumors. Although a negative nuclear reaction for β-catenin cannot exclude a diagnosis of SCT, positive nuclear staining, in our hands, is specific for SCT-NOS, SSCT, and Sertoli–stromal cell tumors among the sex cord–stromal tumors of the testis. This can be helpful, for instance, in the distinction of testicular SCTs from granulosa cell tumors, which can be problematic and oftentimes subjective. The prominent follicle differentiation of juvenile granulosa cell tumors, especially when predominantly of microfollicular type, sometimes is

confused with tubular differentiation of SCTs. Our study, however, showed that all 7 juvenile granulosa cell tumors and 4 adult granulosa cell tumors were negative for β-catenin, making a positive nuclear reaction in this context specific for SCT.

The presence of clear-cut tubular differentiation in SCTs usually distinguishes them from Leydig cell tumors, which are characterized by diffuse growth of tumor cells. However, some SCTs have focal areas of diffuse growth of tumor cells with eosinophilic cytoplasm, which may resemble Leydig cell tumors. Furthermore, occasional Leydig cell tumors show pseudoglandular change that may be confused with tubule formation.[18] Cytokeratin and α-inhibin immunohistochemical stains have been reported helpful but are not definite in differentiating the two tumors.[7] In our study, all 10 Leydig cell tumors were consistently negative for nuclear β-catenin. Therefore, positive nuclear β-catenin stain supports the diagnosis of SCT and argues strongly against the diagnosis of Leydig cell tumor.

Unclassified sex cord–stromal tumor is a heterogeneous group of sex cord–stromal tumors that cannot be adequately classified into a specific group based on morphologic characteristics. Our results showed consistent negative nuclear reaction of β-catenin in this heterogeneous group of tumors, helping to distinguish them from SCTs.

Nuclear positivity for β-catenin is also of assistance in the subtyping of SCTs. All LCCSCTs were negative, making a positive reaction useful in its exclusion from a differential diagnosis. This is a clinically important distinction because of the linkage of the LCCSCT to the Carney complex and is entirely consistent with the pathogenesis of LCCSCT being separate from that of SCT-NOS and SSCT, a concept additionally supported by its association with *PRKAR1A* gene mutation.[3, 19]

Besides its potential diagnostic value for SCTs, our finding also supports the previous experimental and clinical evidences linking β-catenin nuclear accumulation with the pathogenesis of SCTs of the testis. β-catenin is a cell membrane protein that links cadherin adhesion receptors to α-catenin, which in turn links to the cytoskeleton.[8, 20, 21] Cytoplasmic β-catenin interacts with the adenomatous polyposis coli (APC) and axin proteins, which recruit glycogen synthase kinase-3 and casein kinase I to form a destruction complex that rapidly phosphorylates and degrades β-catenin.[22, 23] Little cytoplasmic residue of β-catenin is present under normal condition. During some neoplastic processes, the destruction complex is inhibited and, as a result, the level of free cytoplasmic β-catenin rises and β-catenin enters the nucleus and binds with the T-cell factor/lymphoid-enhancing factor family. This transcriptional complex interacts with the promoters of various target genes affecting cell growth.[24, 25] Laboratory studies have revealed roles of β-catenin in the pathogenesis of SCTs. Conditional stabilization of β-catenin in Sertoli cells caused inhibition of Mullerian duct regression in mice, and overactive β-catenin signaling due to a mutated *CTNNB1* gene was found to cause testicular Sertoli cell tumor development in a mouse model.[26, 27] The pathogenic role of β-catenin in SCTs is supported by results of our study as well as those of Perrone et al, which showed that all SCTs with β-catenin nuclear staining (10/10) had mutations in exon 3 of the *CTNNB1* gene.[15]

One aspect of our study was the high incidence of β-catenin nuclear localization in SSCTs, a finding suggesting a similar pathogenic mechanisms for its formation. Although the SSCT has less frequently displayed malignant behavior compared to SCT-NOS, it may, on occasion, prove malignant.[4, 5] Its relatively favorable prognosis may, therefore, simply be a consequence of its usually smaller size and decreased cellularity compared to SCT-NOS rather

than reflecting a distinctive clinicopathologic entity. The results of this study support that viewpoint.

In summary, results of our study showed that SCT-NOS, SSCT, and Sertoli–stromal cell tumor frequently demonstrate β-catenin nuclear localization, which is not seen in any other type of sex cord–stromal tumor of the testis. β-catenin is, therefore, a useful marker for the diagnosis of SCT and the determination of subtypes of SCTs.

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Figure Legend

Figure 1. Representative H&E and β-catenin immunohistochemical stained sections from Sertoli cell tumor, not otherwise specified (A and B), sclerosing Sertoli cell tumor (C and D), and Sertoli-stromal cell tumor (E and F).