Somatic *PRKAR1A* Gene Mutation in a Nonsyndromic Metastatic Large Cell Calcifying Sertoli Cell Tumor

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Large cell calcifying Sertoli cell tumors (LCCSCTs) are rare testicular tumors, representing <1% of all testicular neoplasms. Almost 40% of patients with LCCSCTs will present in the context of an inherited tumor predisposition condition, such as Carney complex (CNC) or Peutz-Jeghers syndrome. We report the case of a 42-year-old man who had presented with a right testicular mass, and was diagnosed with metastatic LCCSCT. The patient underwent radical orchiectomy, achieving initial remission of his disease. However, lymph node and hepatic metastases were identified. He received chemotherapy without response, and he died of complications of his disease 4 years after the initial diagnosis. Genetic analysis of the tumor and a lymph node metastasis identified a somatic frameshift mutation in the *PRKAR1A* gene (c.319delG, p.E107fs*22). The mutation was predicted to result in premature termination of the PRKAR1A protein and, thus, not be expressed at the protein level, consistent with other *PRKAR1A* nonsense mutations. The patient was extensively screened for signs of CNC, but he had no stigmata of the complex. To the best of our knowledge, the present report is the first of a somatic mutation in the *PRKAR1A* gene shown to be associated with a seemingly sporadic case of LCCSCT. Somatic *PRKAR1A* mutations are rare in sporadic tumors, and it is unknown whether this mutation was causative of LCCSCT in our patient who did not have CNC, or contributed to the malignancy of the tumor, which might have been caused by additional mutations.

Large cell calcifying Sertoli cell tumors (LCCSCTs) represent one of the three known histological types of Sertoli cell-derived sex cord-stromal testicular malignancies [1]. LCCSCTs are rare, accounting for <1% of all testicular neoplasms, and often present in young males, with a mean age at diagnosis of 30 years [2]. They are characterized by hyperplastic Sertoli cells with abundant eosinophilic cytoplasm and surrounding calcifications [3]. LCCSCTs are often considered benign neoplasms, with <17% of reported cases displaying a malignant phenotype and presenting with metastatic disease [2].

Most cases of LCCSCTs have been sporadic (60%); however, 40% of patients with LCCSCTs will present in the context of multiple neoplasia syndromes, such as Carney

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Abbreviations: CNC, Carney complex; LCCSCT, large cell calcifying Sertoli cell tumor; PJS, Peutz-Jeghers syndrome; PKA, protein kinase A; RR, reference range.

complex (CNC) and Peutz-Jeghers syndrome (PJS) [4–6]. CNC is caused by germline inactivating mutations in the *PRKAR1A* gene in 70% of cases; these mutations lead to aberrant cAMP-stimulated protein kinase A (PKA) activity, leading to tumorigenesis in several tissues [7]. PJS is associated with mutations in the *STK11* gene (also known as *LKB1*). The genetic profile of sporadic cases of LCCSCTs is largely unknown, and somatic mutations in *PRKAR1A* gene have not, to the best of our knowledge, been previously reported.

We describe the case of an LCCSCT with a somatic mutation in the *PRKAR1A* gene, without an association with CNC. The patient presented with extensive metastatic disease that was resistant to chemotherapy and other treatments, and died of complications of his disease.

1. Subject and Methods

A. Subject

The patient was evaluated at the Clinical Center at the National Institutes of Health (NIH) under protocol 95-CH-0059. The Eunice Kennedy Shriver National Institute of Child Health and Human Development institutional review board approved the present research. The patient provided written informed consent for all laboratory and imaging studies performed.

B. Immunohistochemistry

Tissue samples from the patient's resected testicular mass and the biopsied lymph node were analyzed. Routine staining with hematoxylin and eosin was performed on several sections across each sample. The monoclonal mouse anti-human Ki-67 (clone MIB1) antibody (1:200 dilution; Agilent, Santa Clara, CA) [8] and mouse anti-human inhibin alpha (clone R1) antibody (1:100 dilution; Bio-Rad, Hercules, CA) [9] were used for immunohistochemistry. The procedures were performed in Ventana Ultra Autostainers (Roche Diagnostics, Indianapolis, IN).

C. Sequencing Analysis

Germline DNA was isolated from peripheral blood lymphocytes using the Maxwell® 16 Blood DNA Purification Kit in a Maxwell® 16 Instrument (Promega, Madison, WI). Tumor DNA was extracted from the primary testicular tumor and the lymph node metastases from paraffin-embedded sections.

The initial genetic evaluation was performed on a specimen from a metastatic lymph node. The lymph node was analyzed using a next-generation sequencing–based assay, including a panel of >300 genes implicated in tumorigenesis, performed as previously described (FoundationOne, ©2017 Foundation Medicine, Inc., Cambridge, MA) [10]. The identified result in the *PRKAR1A* gene was subsequently evaluated with Sanger sequencing on the lymph node, primary tumor, and germline DNA. On the follow-up evaluation of the patient at the NIH. The peripheral blood DNA was further analyzed using whole exome sequencing.

2. Results

A. Clinical Description

The patient presented at 42 years old with a right testicular mass that had been increasing in size for 12 months. He did not have any substantial medical history, including no previous diagnosis of cardiac, adrenal, pituitary, thyroid, or bone lesions. His family history was noncontributory. On physical examination, he did not have any abnormal skin findings. Testicular examination identified a right scrotal mass.

A testicular tumor was confirmed on ultrasonography, and the patient underwent right orchiectomy. The histological evaluation of the mass was consistent with LCCSCT, with a

maximum diameter of 1.6 cm, extending to the rete testis and tunica albuginea, with no involvement of the margins and no lymphovascular invasion, staged as T1N0M0. One year later, an enlarged retroperitoneal lymph node of 3 cm was identified on imaging studies, and retroperitoneal lymph node dissection was performed, revealing metastatic disease in 1 of 17 resected lymph nodes. Approximately 18 months later, the patient presented with new-onset fatigue, radicular pain of the right leg, and weight loss. At further evaluation by imaging, he was found to have extensive metastatic disease, involving the mediastinal and retroperitoneal lymph nodes, the lungs and the liver (Fig. 1).

The patient received two cycles of chemotherapy with etoposide and cisplatin. However, continuous tumor growth was observed, and the therapy was discontinued. Palliative radiation therapy (3000 cGy) was administered to the pelvis and sacroiliac joint without improvement of the pain (not associated with bone metastases). Given the unsuccessful response to the initial chemotherapy protocol, the patient was enrolled in a clinical trial involving the administration of a small molecule tyrosine kinase inhibitor combined with dual immune checkpoint inhibitors. However, progressive growth of the metastatic lesions was observed, and the therapy was discontinued. The patient died of complications of his disease 3 months later. No further biologic material had been obtained beyond the original biopsy specimens.

B. Sequencing Results

Genetic analysis of the lymph node revealed a frameshift mutation in the *PRKAR1A* gene (c. 319delG, p.E107fs*22) that was present in the heterozygote state. Six additional variants of uncertain significance were identified (*CBL*: L43_S44 > HLS, *CHD2*: I593M, *MLL*: R3656Q, *MSH2*: N186S, *MYST3*: G657D, *NOTCH3*: G1710D). No amplification or deletion was identified in the sample.

Sanger sequencing of the PRKAR1A gene of the lymph node confirmed the result in ~18% of LCCSCT cells (Fig. 2), and analysis of the DNA extracted from the testicular tumor (mixed with normal surrounding tissue) showed the presence of the same mutation in a portion of the tumor cells (10% of all cells). Evaluation of germline DNA did not reveal any mutation or deletion/duplication of the *PRKAR1A* gene nor any additional variant of interest (causative or of uncertain significance) in genes involved in tumorigenesis. Although the sequencing results were repeated, with findings consistent with those stated above, no additional tissue could be obtained because the patient's progress was dismal and no other procedures were performed.

The c. 319delG mutation is located in exon 3 of the *PRKAR1A* gene and results in a premature termination codon of the PRKAR1A protein at the amino acid position 129. Prematurely terminating *PRKAR1A* transcripts result in nonsense RNA-mediated decay and are not expressed at the protein level, as we have previously described [4].

C. Immunohistochemistry Results

The tumor cells were positive for inhibin and S-100 but negative for melan A, cytokeratin CAM 5.2, and synaptophysin. The biopsied lymph node showed cells similar to those of the primary tumor but with a higher Ki-67 labeling index, increased cell atypia, and an elevated proliferative rate (Fig. 3).

D. Further Diagnostic Evaluation

Because of the presence of the *PRKAR1A* gene mutation and its association with CNC, the patient underwent extensive biochemical and imaging evaluation to screen for the known findings related to the complex. He did not have any clinical signs of Cushing syndrome. His morning cortisol and ACTH levels were within the normal range (cortisol, 9.7 μg/dL, reference range [RR], 5 to 25; ACTH, 14.4 pg/mL, RR, 5 to 46). No evidence was found of abnormal pituitary hormone secretion, including normal IGF-1 (204 ng/mL, RR, 63 to 279) and prolactin (13.3 μg/dL, RR, 2 to 25) levels. Pituitary MRI did not identify any abnormality of
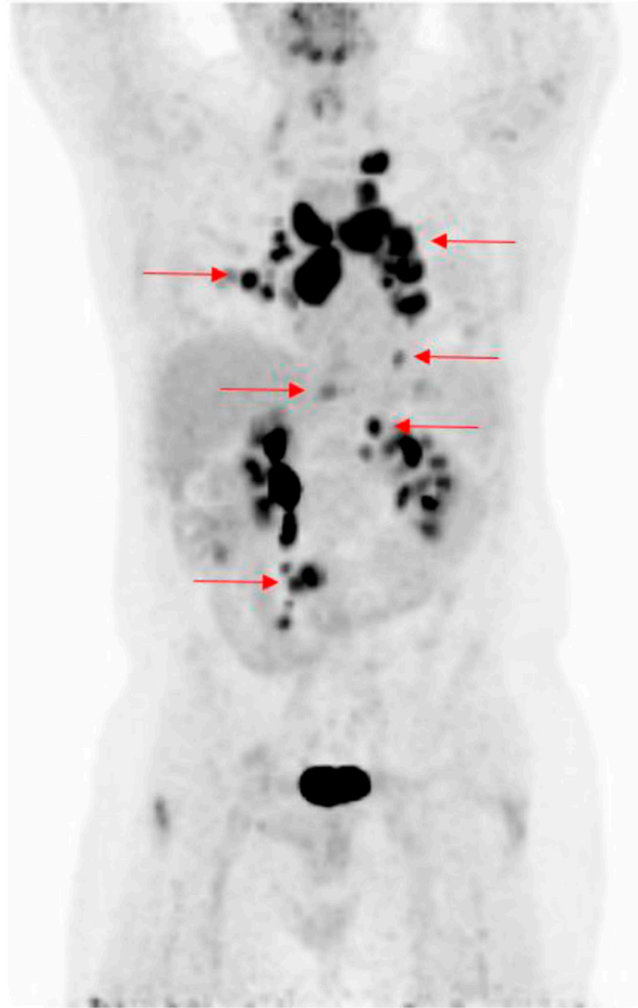
Figure 1. F-18 fluorodeoxyglucose scan of the patient 2 y after the initial diagnosis. Multiple metastatic lesions were present at the mediastinum, lung parenchyma, liver, and para-aortic and retroperitoneal lymph nodes (*arrows*). Physiological F-18 fluorodeoxyglucose activity was present in the head and neck area, kidneys, and urinary bladder.

the pituitary gland. His thyroid function was within the normal range (TSH, 0.86 μIU/mL, RR, 0.27 to 4.2; free T4, 1.2 ng/dL, RR, 0.9 to 1.7), and the thyroid ultrasound scan was only positive for a small homogeneous echopenic lesion of 3×2 mm, of uncertain significance. Finally, an echocardiogram did not reveal any myxomas, and on physical examination he had no skin tags, growth, or other lesions consistent with CNC.

3. Discussion

In the present study, we report the fascinating case of a patient with an isolated, sporadic LCCSCT harboring a somatic mutation in the *PRKAR1A* gene. To the best of our knowledge, the present case is the first case of a somatic *PRKAR1A* defect associated with a sporadic testicular tumor. Although germline *PRKAR1A* gene mutations have been associated with LCCSCTs, no mutations of this gene have been described in sporadic such lesions. Additionally, the patient developed extensive metastatic disease, an uncommon presentation of LCCSCTs, refractory to all lines of treatment, including chemotherapy with cisplatin and etoposide, a tyrosine kinase inhibitor, and immunotherapy. These findings raise the
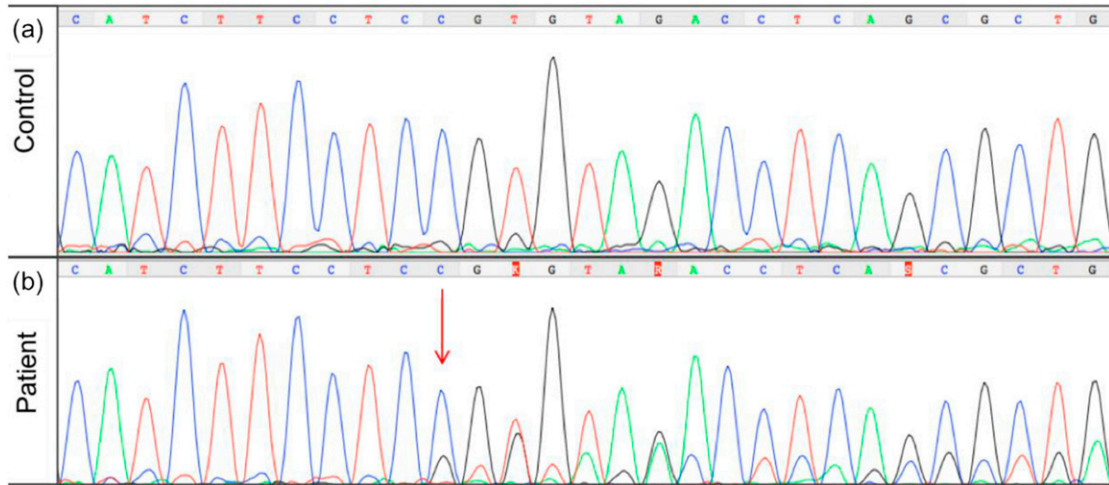
Figure 2. Sequencing analysis of the *PRKAR1A* gene of (a) a control and (b) the patient's lymph node metastasis, in which the mutation c.319delG, p.E107fs*22 (*red arrow*) was present in ~18% of the analyzed DNA.

question of whether the identified genetic cause at the somatic level contributed to a more malignant progress.

PRKAR1A codes for the type 1α regulatory subunit of the PKA tetramer. cAMP, produced after the G-coupled receptor activation of adenylyl cyclase, binds to the regulatory subunits leading to its dissociation from PKA's catalytic subunits. In turn, the catalytic subunits phosphorylate downstream targets, *PRKAR1A*-inactivating mutations result in increased PKA activity by allowing for unregulated and unbound catalytic subunits [11]. Somatic
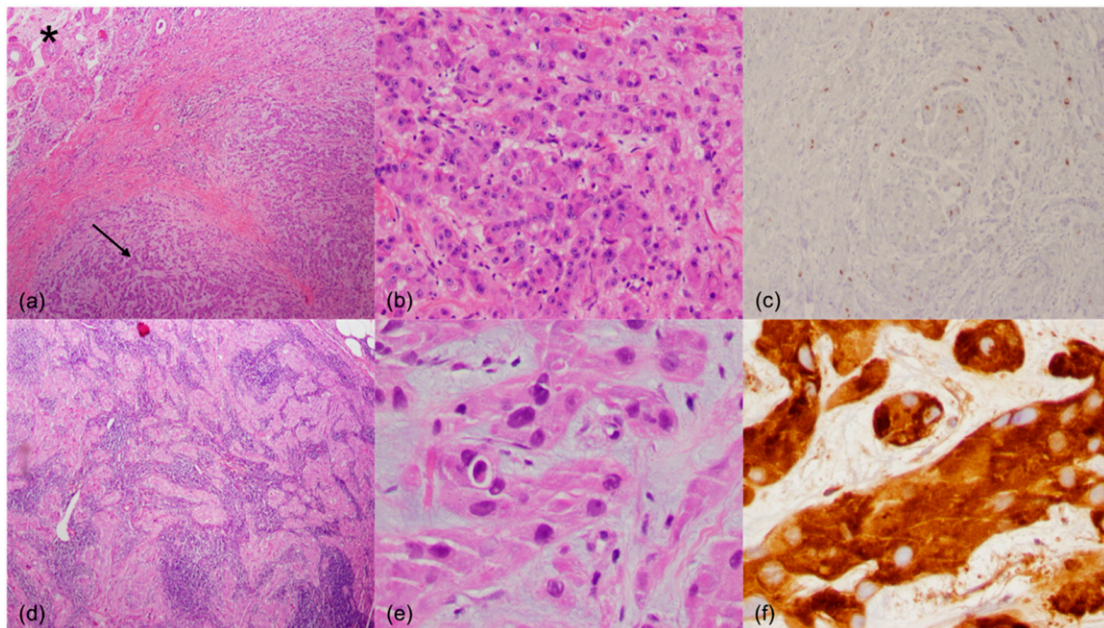

Figure 3. Staining and immunohistochemistry images of the (a-c) resected primary tumor and (d-f) peripheral lymph node with metastasis. (a, b) Sections show a testicular neoplasm composed of nests and cords of large tumor cells with eosinophilic cytoplasm and prominent nucleoli in a background of fibrotic stroma. Mild cell atypia was present, but mitoses or necrosis were not readily visible. (c) The proliferative index as per Ki-67 staining was ~5%. (d, e) Tissue from metastatic LCCSCT had morphology similar to that of the primary lesion and was positive for inhibin alpha staining (f). *Asterisk,* normal testis; *arrow,* LCCSCT; Magnification, 4× (a, d, c), 10× (b), and 40× (e, f).

mutations of the *PRKAR1A* gene have been implicated in the pathogenesis of several sporadic tumors such as cardiac myxomas, adrenal adenomas, thyroid and adrenal cancer [12–14]. Somatic dysregulation of *PRKAR1A* expression may also be implicated in other tumors [15].

Germline mutations in the *PRKAR1A* gene are responsible for 70% of cases of CNC, although a few patients will harbor defects at another locus on chromosome 2p16 [7, 11]. The major clinical characteristics of CNC include skin findings (spotty skin pigmentation, blue nevi, cutaneous or skin myxomas), cardiac lesions (myxomas), endocrine abnormalities (primary pigmented nodular adrenocortical disease, GH-producing adenomas, thyroid gland lesions), breast lesions (myxoid fibroadenomas, ductal adenomas), peripheral nerve tumors (psammomatous melanotic schwannomas), and bone tumor (osteochondromas) [16]. Additionally, LCCSCTs in males and ovarian abnormalities in females are often encountered in association with CNC, in 41% and 14% of cases, respectively [17–19].

Approximately 40% of LCCSCTs will be related to hereditary conditions such as CNC and PJS. They often appear in young patients (mean age, 17 years) and are usually bilateral and multifocal [2]. Most importantly, they appear to be benign and can be treated with aromatase inhibitors alone or with radical or partial orchiectomy if they produce estrogen hormones [20]. In the remaining 60% of the cases, the mean age of onset has been 39 years, and the tumors have usually been monofocal and unilateral. The prognosis of these patients seems to be worse, because 23% of them will present with metastases. These tumors can be treated with radical or partial orchiectomy, and patients with retroperitoneal metastases are often advised to undergo retroperitoneal lymph node dissection. However, few patients have experienced a good response to chemotherapy or radiation therapy [2]. The relationship between the patient's age and the tumor's behavior can be highlighted by the finding that only one case of malignant LCCSCT has been described in a prepubertal-aged patient [21].

Genetic testing of sporadic LCCSCTs has not revealed an etiology in most cases. Petersson *et al.* [22] described the case of one patient with a frame shift mutation of the *PRKAR1A* gene who had presented with a benign LCCSCT. Although that patient did not have clinical findings of CNC, no data on the germline status of the *PRKAR1A* gene were reported.

In our patient, several lines of evidence strongly support that the identified mutation was pathogenic. It is a frameshift mutation of *PRKAR1A* gene that leads to the addition of 13 altered amino acids before the formation of a premature stop codon at the amino acid position 129. Mutations in the same amino acid (c.319G>T p.E107X) and mutations leading to termination of the protein at the same (c.340delG, p.V114Lfs*15) or neighboring positions have been previously described in patients with CNC (data from the *PRKAR1A* mutation database; available at: https://prkar1a.nichd.nih.gov/hmdb/mutations.html) [23]. In these cases, just as in most CNC-causing *PRKAR1A* defects, the transcribed mRNA is degraded by nonsense mediated decay [19]. In most patients with CNC and benign LCCSCTs, a mutation leading to a premature stop codon formation will be detected [24]. Most likely, in our patient, too, the mutation produced *PRKAR1A* mRNA was degraded by nonsense mediated decay. Although in certain cases of CNC, biallelic inactivation of the *PRKAR1A* gene has been reported, this is not necessary in all cases or in sporadic tumors (as in our patient) [25]. This implies that monoallelic inactivation of the gene may be sufficient for the pathogenesis of at least some of these tumors or that additional genetic factors might be potentially involved in this process.

In conclusion, we present a case of a malignant metastatic LCCSCT associated with a somatic *PRKAR1A* pathogenic gene mutation detected in both the primary tumor and the metastatic tissue. Although patients with germline *PRKAR1A* mutations are known to present with LCCSCTs, to the best of our knowledge, a somatic defect in a testicular tumor has not been previously described.

Institute Medical Oncology Program, Genitourinary Malignancies Branch, and Dr. Andrea B. Apolo and her team for offering treatment to our patient during the last phases of his disease.

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