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Primary Renal Sarcomas with *BCOR-CCNB3* Gene Fusion: A Report of Two Cases Showing Histologic Overlap with Clear Cell Sarcoma of Kidney, Suggesting Further Link Between *BCOR*-related Sarcomas of the Kidney and Soft Tissues

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Abstract

We report two primary renal sarcomas demonstrating *BCOR-CCNB3* gene fusions that have recently been identified in undifferentiated round cell sarcomas of bone and soft tissue. These neoplasms occurred in male children aged 11 and 12, and both were cystic as a result of entrapment and dilatation of native renal tubules. Both cases were composed of variably cellular bland spindle cells with fine chromatin set in myxoid stroma and separated by a branching capillary vasculature. Both neoplasms demonstrated immunoreactivity for BCOR, cyclin D1, TLE1 and SATB2 in the spindle neoplastic cells and negativity in the prominent capillary vasculature. One case was extensively cystic and had hypocellular areas that simulated cystic nephroma; this neoplasm recurred 3 years later as a solid, highly cellular spindle cell sarcoma in the abdominal cavity. The morphology and immunoprofile of these renal neoplasms was compared to a control group of other sarcomas with *BCOR* genetic abnormalities, including clear cell sarcoma of the kidney (CCSK), infantile undifferentiated round cell sarcomas of soft tissue/primitive myxoid mesenchymal tumor of infancy (URCS/PMMTI), and bone/soft tissue sarcomas with *BCOR-CCNB3* gene fusion; along with primary renal synovial sarcoma. Our findings show that the renal sarcomas with *BCOR-CCNB3* gene fusion overlap with CCSK. They are in keeping with a "*BCOR*-alteration family" of renal and extra-renal neoplasms which includes CCSK and URCS/PMMTI (which typically harbor *BCOR* internal tandem duplication), and *BCOR-CCNB3* sarcomas, all of which are primarily driven by *BCOR* overexpression and have overlapping (but not identical) clinicopathologic features.

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Keywords

Renal Neoplasm; Clear cell sarcoma: BCOR; Translocation

Introduction

Clear cell sarcoma of the kidney (CCSK) comprises approximately 3% of pediatric renal neoplasms, and occurs at a mean patient age of 3 years[1, 2]. The classic morphologic pattern of CCSK is that of nests or *cords* of neoplastic cells separated by regularly spaced, arborizing fibrovascular *septa*. The *cord cells* can be either round, epithelioid or spindled, and are separated by extracellular mucopolysaccharide that creates a resemblance to clear cytoplasm. The nuclei are round to ovoid with fine chromatin without prominent nucleoli. The *septal cells* are spindled, fibroblast-like cells that surround thin, regularly-branching capillaries, and quite often form cellular perivascular sheaths ("cellular septa"). CCSK may also display a variety of variant morphologic patterns that mimic other neoplasms, including myxoid, sclerosing, cellular, spindled, storiform, palisaded, trabecular, and epithelioid patterns. While cyclin D1[3, 4] and SATB2[5] are often immunoreactive in CCSK, most other immunohistochemical markers such as CD34, S100, desmin, CD99, and cytokeratin are negative.

As implied by its name, CCSK has long been thought to be a kidney-specific sarcoma, with only rare putative extrarenal CCSK-like neoplasms reported[1]. Long a mystery, genetic alterations underlying CCSK have been delineated in the past decade. The majority (>90%) of CCSK harbor internal tandem duplications (ITD) in the last exon of the *BCOR* (*Bcl6* interacting co-repressor) gene, which in CCSK is thought to regulate gene transcription through an epigenetic silencing mechanism[6,7,8]. A smaller subset of CCSK harbor a *YWHAE-NUTM2B* gene fusion resulting from a t(10;17)(q22.3;p13.3) translocation which is identical to that seen in high grade endometrial stromal sarcoma[9,10]. These two genetic alterations in CCSK appear to be mutually exclusive[11, 12]. A small subset of otherwise typical CCSK (<10%) lack either alteration[11].

In the past five years, upregulation of BCOR by similar mechanisms as found in CCSK has been identified as an underlying genetic alteration in several soft tissue sarcomas of young patients. First, *BCOR* ITD and the *YWHAE-NUTM2B/E* gene fusion have been identified in undifferentiated round cell sarcoma (URCS) and primitive myxoid mesenchymal tumor of infancy (PMMTI), two neoplasms that typically affect children under the age of 1 year[5, 13, 14]. On careful morphologic review, the latter two neoplasms overlap significantly morphologically with CCSK[14]. Second, a *BCOR-CCNB3* gene fusion resulting from an X chromosomal pericentric inversion has been identified in previously unclassified soft tissue and bone sarcomas which typically affect teenagers and young adults with a male predominance[15–18]. While the majority of these *BCOR-CCNB3* sarcomas were identified among *EWSR1*-negative Ewing-sarcomas and thought to be primarily small round neoplasms[15, 16], more recently cases with spindled morphology have been described[17, 18]. Importantly, high levels *BCOR* mRNA expression resulting in BCOR protein overexpression as detected by immunohistochemistry[5] and a distinctive *BCOR*-driven transcriptional

profile[14] have been identified in URCS/PMMTI, *BCOR-CCNB3* fusion-positive sarcomas, and CCSK, suggesting that all of these neoplasms are highly genetically related. However, the existence of *BCOR-CCNB3* sarcomas originating in the kidney as well as a relationship between bone/soft tissue sarcomas with the *BCOR-CCNB3* fusion and CCSK has not previously been described.

In this report, we describe two primary renal sarcomas harboring *BCOR-CCNB3* gene fusions. We review the morphology and perform a broad immunochemical analysis of these neoplasms, and compare them to control groups of typical CCSK, *BCOR-CCNB3*–positive bone/soft tissue sarcoma, and *BCOR*-ITD positive URCS/PMMTI. Review of the morphology and immunohistochemical profile of *BCOR-CCNB3* –positive renal and bone/soft tissue sarcomas demonstrates overlap with CCSK. Finally, given prior work demonstrating upregulation of BCOR identified in approximately 50% of soft tissue synovial sarcomas, we examine the expression of BCOR and related markers in primary renal synovial sarcoma[19], which represents the main differential diagnosis of the *BCOR-CCNB3* renal sarcomas.

Methods

Cases

Both renal sarcomas with *BCOR-CCNB3* gene fusion were identified in a review of renal sarcomas in our files with overlapping features of CCSK and renal synovial sarcoma. Both of these neoplasms had been considered unclassified at the time of diagnosis. All of the cases in the review cohort were screened by immunohistochemistry by BCOR, and positively-labeling cases were analyzed for the presence of *BCOR* and *CCNB3* fusion/inversion by FISH. Break-apart FISH was also performed with custom BAC probes for the following genes: *SS18, SSX1, SSX2, SS18L1* (all for synovial sarcoma), or *YWHAE* (for CCSK) using previously described methodology[20]. *BCOR* and *CCNB3* FISH probes utilized are listed in Supplementary Table 1. *SSX1*, *SSX2*, *SS18* and *SS18L1* probes utilized have been described previously[20].

Review of a control group of BCOR-associated soft tissue sarcomas

Following identification of renal sarcomas with *BCOR-CCNB3* gene fusions, we reviewed the morphologic and immunohistochemical features of a cohort of 20 undifferentiated round cell sarcomas/primitive myxoid mesenchymal tumors of infancy (URCS/PMMTI) (all showing *BCOR* internal tandem duplication) and 3 other cases demonstrating the *YWHAE-NUTM2B/E* fusion from the files of one author (CRA), most of them included in previous publications[5,14]. All but one of these neoplasms had accompanying BCOR IHC for evaluation. We also reviewed 24 cases of genetically confirmed *BCOR-CCNB3* bone and soft tissue sarcomas from the files of one author (CRA). Twelve of these cases had accompanying BCOR IHC.

Primary Renal Synovial Sarcoma Control Group

We identified 7 genetically confirmed primary renal synovial sarcomas from our files, all of which demonstrated *SS18* rearrangements by FISH. These cases occurred in 5 males and 2

females. These patients ranged in age from 35 to 66 (mean age 49 years; median age 48.5 years). Of cases with known fusion partner, 3 of 4 were *SSX2* while one was *SSX1*, consistent with the previously-reported distribution of gene fusions found in primary renal synovial sarcoma[21].

Clear Cell Sarcoma of Kidney (CCSK) Control Group

We identified 9 CCSK from the files of one author (PA). These cases occurred in 6 males and 3 females, and ranged in age from 6 months to 42 months (mean 24 months, median 19 months). All 9 of these cases demonstrated strong nuclear labeling for BCOR by immunohistochemistry. Of the 5 cases studied genetically, 4 demonstrated *BCOR* internal tandem duplication (ITD), while one demonstrated the *YWHAE-NUTM2B* gene fusion.

Immunohistochemistry was performed as previously described[1, 5, 14, 19] for the following proteins on the *BCOR-CCNB3* renal sarcomas: BCOR, SATB2, TLE1, cyclin D1, Bcl2, CD56, CD99, CD10, desmin, S100, cytokeratin AE1/3 and, cyclin D1. Immunohistochemistry for selected markers from this group were also performed on cases in the control groups.

Results

Case Reports

Case 1 was an 11 year-old male who presented with a 27 cm cystic renal neoplasm which was treated by radical nephrectomy. The nephrectomy specimen weighed 1,845 grams and no further follow-up is available.

Case 2 was a 12 year-old male who presented with a 13 cm solid and cystic renal neoplasm which was treated by radical nephrectomy. Fifteen months later, the patient developed an abdominal mass which represented a recurrence of the prior neoplasm.

In both cases, the primary renal neoplasm was extensively cystic, with the cysts being lined by non-neoplastic, dilated native renal tubules. The cyst walls were variably cellular in both cases. In many areas of case 1, the cyst walls were composed of non-descript, non-pleomorphic, bland spindle cells which condensed beneath the cyst epithelium, creating a “cambium-like” appearance. In other areas, these cells became more plump and epithelioid, and alternated with prominent perivascular spindle cells in a pattern reminiscent of the biphasic cord cell-septal pattern of CCSK. The more epithelioid cell nuclei were bland, and had fine, open chromatin similar to that of CCSK (Figure 1).

In case 2, the cystic primary renal neoplasm was extremely bland. The cyst walls were hypocellular and composed predominantly of uniform spindle cells with minimal cytoplasm, creating a resemblance to cystic nephroma or mixed epithelial stromal tumor. Focally, the cyst walls were slightly more cellular, and one could appreciate a biphasic cord cell/septal cell pattern that suggested CCSK. The neoplastic cells in the cyst walls were non-pleomorphic and lacked mitotic activity (Figure 2). The abdominal recurrence of this neoplasm, in contrast, demonstrated solid fascicles of cellular, non-pleomorphic spindle cells with frequent mitotic figures.

Both cases demonstrate similar immunohistochemical profiles; therefore, they are discussed together. In both cases, the neoplasm demonstrated strong and diffuse nuclear labeling for BCOR. Importantly, BCOR IHC highlighted the biphasic pattern of these lesions, with strong nuclear labeling of the "cord cells" and absence of labeling in the prominent admixed "septal cells", similar to the pattern previously described in CCSK (Figure 1E, 2E)[5]. As suggested by the morphology, the BCOR immunoreactive cells were less frequent and focally absent in the bland, hypocellular cyst walls of the primary cystic renal neoplasm in case 2, but comprised the majority of the cells in the solid, high grade spindle cell recurrence of that lesion (Figure 2H). Both neoplasms also demonstrated diffuse immunoreactivity for Bcl2, CD56, SATB2, cyclin D1, and TLE1 (Figure 1H, 2F). Desmin, S100 protein, cytokeratin AE1/3, and CD34 were negative in both cases. PAX8 was negative in both the primary and recurrent tumor in case 2 (native renal tubules in the primary were PAX8 positive, providing a positive internal control). In case 1, there was focal weak nuclear staining of the more epithelioid "cord cells", but this was less intense than that of the entrapped native renal tubules that provided an internal control (Figure 1G).

FISH demonstrated an inversion-fusion pattern between *BCOR* and *CCNB3* in both cases, including both the bland cystic primary renal neoplasm in case 2 and its high grade abdominal recurrence (Figure 3).

Review of the control group of BCOR-related soft tissue sarcomas

The control group of 23 URCS/PMMTI soft tissue cases with either *BCOR* ITD or *YWHAE* fusions occurred in patients ranging from ages 2 weeks to 14 months. As previously described, these neoplasms overlap significantly with CCSK, in that they featured bland round, epithelioid to spindled "cord cells" with open chromatin and prominent "septal cells"[14]. There was strong nuclear labeling for BCOR in 21 of the 22 cases in which immunohistochemistry (IHC) was available. Importantly, the BCOR IHC highlighted the "cord cells" but not the "septal cells", similar to the pattern noted in renal CCSK as previously noted[5]. Also of note, 1 case was predominantly composed of a high grade spindle cell component more typical of the *BCOR-CCNB3* sarcomas, while focal areas of spindling were noted in one third of cases[14].

The 24 *BCOR-CCNB3* bone and soft tissue sarcomas occurred in patients ranging in age from 2 years to 24 years. On review, all demonstrated undifferentiated round to spindle cell features that resembled the cellular spindle pattern of CCSK[1], in which the "cord cells" acquire a more spindled appearance that resembles monophasic spindle cell synovial sarcoma[1]. BCOR immunohistochemistry highlighted the prominent labeling of "cord cells" and absence of labeling in the prominent "septal cell" component, as previously demonstrated in CCSK[5], in all 12 cases in which it was performed (Figure 4). Also of note, a subset of bone/soft tissue cases with the *BCOR-CCNB3* fusion demonstrated focally low grade myxoid areas which overlapped with URCS/PMMTI and the myxoid, hypocellular pattern of CCSK.

Of note, in one case PAX8 immunohistochemistry had been performed in a primary soft tissue *BCOR-CCNB3* sarcoma, and demonstrated weak labeling in cord cells similar to that seen in renal *BCOR-CCNB3* case 1 (Figure 4G).

Six of seven *BCOR-CCNB3* bone/soft tissue sarcomas and all four URCS/PMMTI tested were immunoreactive for cyclin D1. Seven of nine *BCOR*-ITD URCS/PMMTI and five of nine soft tissue *BCOR-CCNB3* sarcomas were immunoreactive for CD99, though typically in a diffuse cytoplasmic pattern rather than a strong membranous distribution. Both *BCOR-CCNB3*–positive bone/soft tissue sarcomas tested demonstrated immunoreactivity for TLE1.

Clear cell sarcoma of the kidney (CCSK) (control group)

All 9 CCSK cases demonstrated typical morphologic features, specifically cord cells with fine chromatin and indistinct cytoplasm set in a myxoid stroma and separated by a regular branching capillary vasculature lined by septal cells. All nine cases demonstrated strong diffuse nuclear labeling for BCOR in the cord cells. As expected from prior literature, three of seven cases studied (42%) demonstrated diffuse nuclear labeling for SATB2, and all five tested showed diffuse nuclear labeling for cyclin D1. Four of 5 tested cases demonstrated strong diffuse nuclear labeling for TLE1, as marker not previously studied in CCSK (Figure 5). One of 5 tested CCSK demonstrated strong, diffuse labeling for PAX8 (Figure 6).

Primary renal synovial sarcoma (control group)

The seven genetically confirmed primary renal synovial sarcomas were composed of cellular non-pleomorphic spindle cells associated with dilated native renal tubules in four cases, typical of monophasic spindle cell synovial sarcoma arising in the kidney[19]. Of these seven cases, four demonstrated nuclear immunoreactivity for BCOR (58%). As expected, four of five cases tested demonstrated strong diffuse nuclear labeling for TLE1, while three of five cases tested were positive for cyclin D1. Two of five cases tested demonstrated focal weak/equivocal staining for SATB2; the other three were completely negative.

Discussion

We report two primary renal sarcomas demonstrating *BCOR-CCNB3* gene fusion. In the absence of molecular findings, these cases were originally considered to be unclassified sarcomas with a differential diagnosis including CCSK and primary renal synovial sarcoma. As neoplasms with *BCOR-CCNB3* gene fusion, similar to sarcomas with *BCOR*-ITD, are associated with a *BCOR*-driven transcriptional profile, we interrogated the possible relationship of these neoplasms to CCSK. These cases were somewhat unusual for CCSK, in that they affected slightly older children (ages 11 and 12) compared to the mean CCSK age of 3, demonstrated predominant spindle morphology, and were associated with extensive dilation of native renal tubules resulting in extensive cystic change. In one case, the combination of hypocellular neoplastic cells within the septa and extensive cystic change created a mimic of a benign mixed epithelial stromal tumor or cystic nephroma. However, while somewhat unusual, the *BCOR-CCNB3* renal sarcomas are certainly compatible with CCSK. The patient ages of the *BCOR-CCNB3* renal sarcomas are well within the spectrum of CCSK, which in the largest study in the literature (351 cases) occurred in an age range of 2 months to 14 years[1]. Predominant spindle cell morphology and extensive cystic change mimicking cystic nephroma, as classically seen in renal synovial sarcoma, have also previously been described and illustrated in CCSK[1]. We found that the *BCOR-CCNB3* renal sarcomas expressed TLE1, a sensitive marker of synovial sarcoma[22,23] which had not

previously been studied in CCSK: however, we found that TLE1 was also positive in the typical CCSK in our control group. Both *BCOR-CCNB3* renal sarcomas labeled for BCOR, cyclin D1 and SATB2 which are recognized markers of CCSK. Overall, taking together the common morphologic features of these neoplasms including the biphasic BCOR-immunopositive cord cells and BCOR-immunonegative septal cells, along with the immunoreactivity for cyclin D1, TLE1 and SATB2 in the cord cells in the absence of labeling for CD99, desmin, cytokeratin, S100 and CD34, the pathologic features fall within the broad spectrum of CCSK.

Despite their common transcriptional profile driven by a consistent upregulation of *BCOR* mRNA expression, some differences exist between soft tissue undifferentiated round cell sarcoma (URCS)/primitive myxoid mesenchymal tumor of infancy (PMMTI), which harbors *BCOR* internal tandem duplication (ITD), and *BCOR-CCNB3* bone and soft tissue sarcomas. First, CCNB3 overexpression both at the mRNA[5,14] and protein[15] levels is seen only in *BCOR-CCNB3* bone/soft tissue sarcomas and not in URCS/PMMTI with *BCOR* ITD. Second, the age at presentation is different; URCS/PMMTI almost overwhelmingly occur in infants, while *BCOR-CCNB3* soft tissue sarcomas typically affect teenagers and young adults. Third, most URCS/PMMTI have a heterogeneous appearance with alternating compact round cellular areas with hypocellular myxoid components, whereas *BCOR-CCNB3* sarcomas are uniformly highly cellular, rounded and often spindled neoplasms which overlap with Ewing sarcoma and synovial sarcoma. Despite these differences, our review of a large series of URCS/PMMTI and *BCOR-CCNB3* sarcomas of bone and soft tissue found significant areas of overlap. Specifically, PMMTI-like areas can be seen at the edge of *BCOR-CCNB3* undifferentiated sarcomas of bone and soft tissue, and URCS/PMMTI often have higher grade areas that overlap with *BCOR-CCNB3* sarcomas. Previous studies from our group have highlighted morphologic similarities between the URCS/PMMTI harboring *BCOR* ITD and CCSK[5]. In this study, we note the similarity of *BCOR-CCNB3* sarcomas of bone/soft tissue and kidney with CCSK. This is highlighted by presence of cellular septa within the *BCOR-CCNB3* soft tissue sarcomas which are very similar to those seen within CCSK. In both instances, the cellular septa do not label for BCOR, suggesting that these cellular septa represent a florid pericyte-rich reactive proliferations associated with these *BCOR-CCNB3* driven neoplasms. Hence, just as in soft tissue where the *BCOR-CCNB3* sarcomas overlap with the URCS/PMMTI with *BCOR* ITD, the primary renal sarcomas with the *BCOR-CCNB3* gene fusion overlap with CCSK showing *BCOR* ITD (Table 1). We feel that minor differences between CCSK and the *BCOR-CCNB3* renal sarcomas are less significant than their unifying features. However, greater experience with the clinicopathologic features and response to therapy of the *BCOR-CCNB3* renal sarcomas are needed to further establish this link. We note that a single neoplasm with the *BCOR-CCNB3* gene fusion was recently reported in abstract form within a series reported as CCSK[24]. We propose that CCSK, URCS/PMMTI, and *BCOR-CCNB3* sarcomas of kidney and bone/soft tissue can be thought of as a "*BCOR*-alteration family" of renal and extra-renal neoplasms with a common genetic signature and overlapping (but not identical) clinicopathologic features (Table 2).

The focal PAX8 labeling of *BCOR-CCNB3* renal sarcoma case 1 might be considered unusual for CCSK, which has previously been considered negative for PAX8 though PAX2

labeling has been reported[25]. However, we found that PAX8 is positive in a minority of CCSK, and can be focally expressed in soft tissue *BCOR-CCNB3* sarcomas. Furthermore, a recent study found that PAX8 is expressed in over 50% of BCOR-CCNB3 soft tissue sarcomas[26], and PAX8 immunoreactivity also been described in primitive round cell sarcomas such as alveolar rhabdomyosarcoma and rhabdoid tumor[25]. Hence, in the setting of primitive round/spindle cell neoplasms, PAX8 is not specific for renal parenchymal versus mesenchymal origin.

The predominantly spindle appearance of the *BCOR-CCNB3* renal sarcomas created significant overlap with synovial sarcoma, which was the main differential diagnosis for these cases. Morphologic and immunohistochemical overlap of *BCOR-CCNB3* soft tissue sarcomas with synovial sarcoma has been reported[27], as has overlap between CCSK and renal synovial sarcoma[28]. Along these lines, BCOR overexpression has been demonstrated in approximately half (49%) of soft tissue synovial sarcomas[5], though BCOR expression had not been addressed in primary renal synovial sarcomas until now. In this study, we document that primary renal synovial sarcomas similarly overexpress BCOR in approximately half (60%) of cases. Primary renal synovial sarcomas also express cyclin D1, a sensitive marker of CCSK, and TLE1, a sensitive marker of soft tissue synovial sarcoma, furthering the potential overlap with *BCOR-CCNB3* renal sarcoma. As suspected based on previously reported expression profiling and immunohistochemical analysis of soft tissue synovial sarcoma[5], SATB2 is less frequently expressed in renal synovial sarcoma than in typical CCSK or the renal *BCOR-CCNB3* sarcomas, providing one useful discriminatory immunohistochemical marker. While analysis of *BCOR* gene status and the *SS18-SSX* gene fusions typically resolve the differential diagnosis of CCSK and renal synovial sarcoma, there may also be significant overlap at the genetic level. Along these lines, a case of a poorly differentiated synovial sarcoma with a variant *SS18L1-SSX1* gene fusion and complex rearrangements affecting the Xp11.22-4 region including disruption of *BCOR* and upregulation of BCOR protein has been reported[20].

Our finding of overlap between *BCOR-CCNB3* fusion-positive sarcomas of the kidney and soft tissue with CCSK has potential therapeutic implications. *BCOR-CCNB3* sarcomas of bone and soft tissues were initially recognized as a subset of *EWS*-non-rearranged “Ewing sarcomas”, and have historically been treated with Ewing sarcoma chemotherapy regimens, though some evidence exists that their behavior may be more indolent[17]. As such, *BCOR-CCNB3* sarcomas are typically treated as Ewing sarcomas at most centers today. Given the overlapping morphology of *BCOR-CCNB3* sarcomas which CCSK and their overlapping transcriptional profile, and given that patients with CCSK have been shown to benefit from specific doxorubicin-based chemotherapy[1, 29], it seems logical to consider treating the *BCOR-CCNB3* soft tissue sarcomas with CCSK-based therapy regimens (which emphasize Doxorubicin and do not include Ifosfamide) rather than Ewing sarcoma-based regimens (which include both Doxorubicin and Ifosfamide). The former chemotherapy regimen is overall less toxic, and at least in CCSK has demonstrated significant clinical benefit.

In summary, we report two primary renal sarcomas with the *BCOR-CCNB3* gene fusion. We demonstrate that while some features are unusual for CCSK, these neoplasms do overlap with CCSK at the morphologic, immunohistochemical, and genetic level. *BCOR-CCNB3*

renal sarcomas may account for a subset of cases currently classified as CCSK but which lack *BCOR*-ITD or *YWHAE-NUTM2B/E* gene fusions. Our findings support the concept of a *BCOR*-alteration family of renal and extrarenal neoplasms having a highly related genetic profile and similar (though not identical) clinicopathologic features, including CCSK, *BCOR-CCNB3* sarcomas, and undifferentiated round cell sarcoma/primitive myxoid mesenchymal tumor of infancy (URCS/PMMTI).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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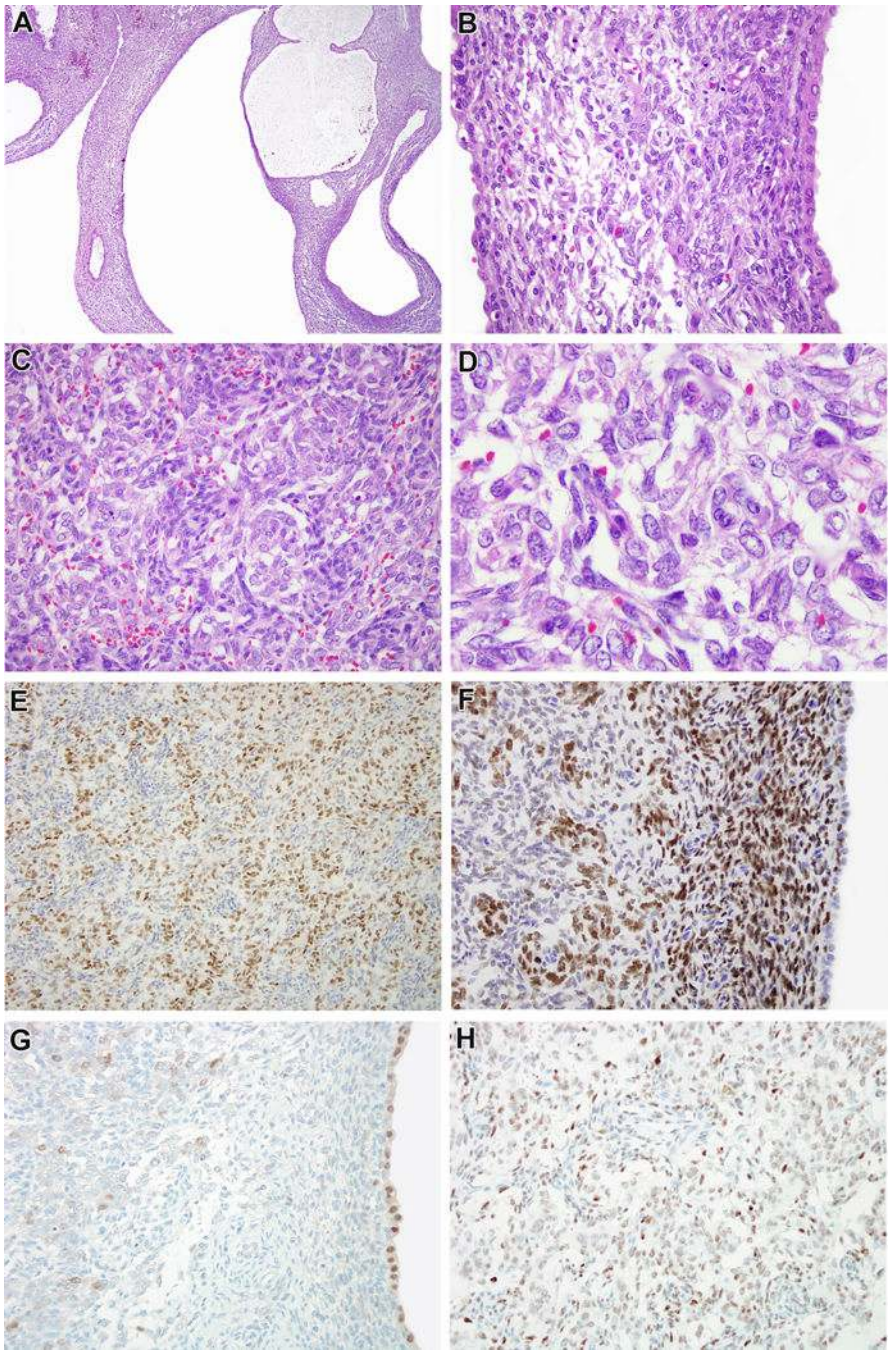

Figure 1.

Case 1. This 27 cm renal neoplasm was extensively cystic (A). The cysts were lined by bland cuboidal epithelial cells with eosinophilic cytoplasm consistent with native renal tubules, while the stroma was moderately cellular, non-pleomorphic, and demonstrated subepithelial condensations resembling a cambium layer (B). In more cellular areas, one could appreciate a biphasic cord cell-septal cell appearance similar to that seen in clear cell sarcoma of the kidney (CCSK). A regular branching capillary vasculature was more evident in these areas. The cord cells demonstrated finely dispersed, open chromatin, particularly

relative to the more hyperchromatic septal cells (D). Immunohistochemistry for BCOR highlighted the cord cells (E), and highlighted the cambium layer but not the entrapped cyst lining (F). Focally, the cord cells demonstrated weak labeling for PAX8 relative to the intense staining of the cyst lining (G). The cord cells were immunoreactive for SATB2 (not shown) and TLE1 (H).

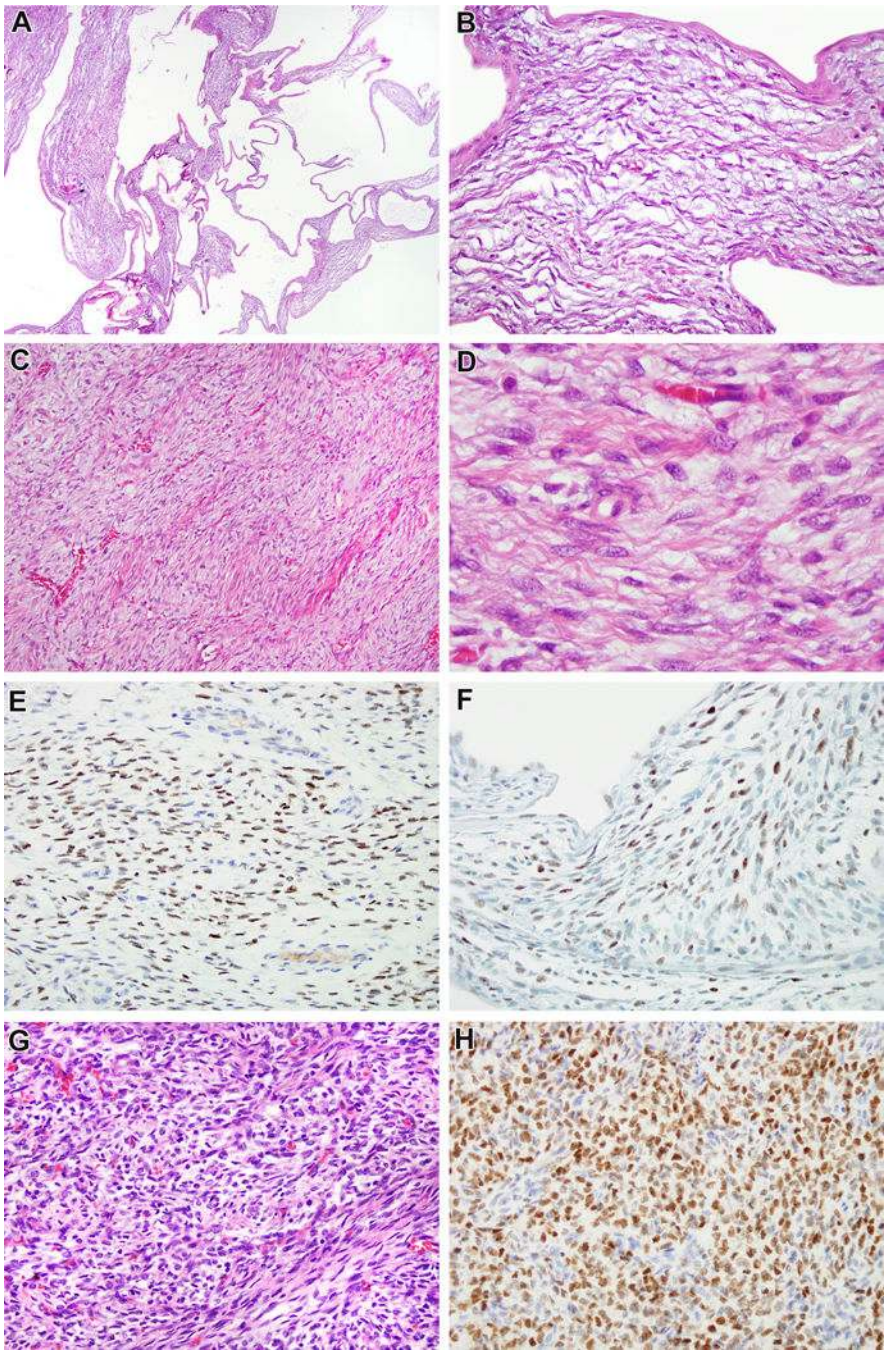

Figure 2.

Case 2. The original 13 cm renal neoplasm was extensively cystic (A) and the septa were relatively hypocellular in most areas (B), raising the differential diagnosis of cystic nephroma. Focally, more cellular areas in the septa demonstrated the branching capillary vasculature that suggested CCSK (C) and the cord cells between septa demonstrated bland, finely dispersed chromatin (D). The cord cells demonstrated nuclear labeling for BCOR (E), SATB2 (not shown) and TLE1 (F). The abdominal recurrence 15 months later was a highly cellular non-pleomorphic spindle cell neoplasm which again had a suggestion of biphasic

cord cell-septal cell appearance that is characteristic of CCSK (G). The cord cells demonstrated diffuse immunoreactivity for BCOR while the septal cells did not (H), highlighting this distinction.

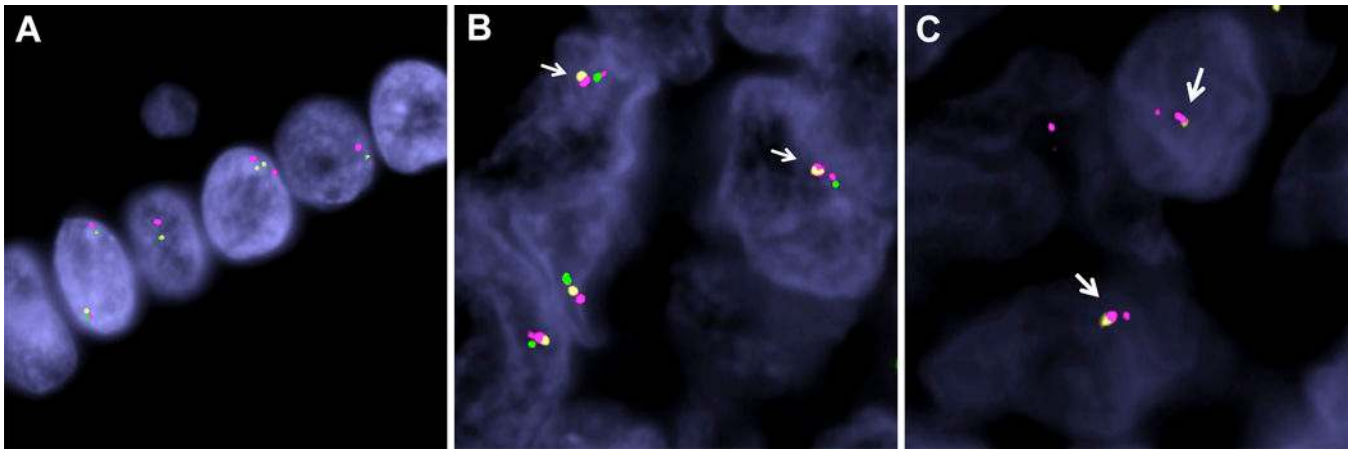

Figure 3.

Demonstration of *BCOR-CCNB3* gene fusion by FISH. A. Normal cells analyzed with FISH probes. The green-orange probes represent *BCOR* and the red flanking probe represents *CCNB3*. Note the normal, small gap between the two genes, which corresponds to 9MB. B. Case 1. The two neoplastic cells illustrated show a *BCOR-CCNB3* fusion/inversion. The *CCNB3* (red) shows a split signal into a larger fragment (centromeric *CCNB3*) and a smaller signal (telomeric *CCNB3*); while the *BCOR* shows a break of the green (telomeric) and orange (centromeric) signals. The end-result is a fusion between the centromeric *BCOR* signal (orange) to the centromeric *CCNB3* (larger red) (arrows), along with fusion of telomeric *BCOR* signal (green) to telomeric *CCNB3* signal (smaller red). C. Case 2. The two neoplastic cells illustrated show *CCNB3* inversion reflected by the red probe split into two signals (larger, centromeric and smaller, telomeric) and a *BCOR* unbalanced break, with deletion of telomeric end (no green signal). The resulting fusion is composed of centromeric 5′ *BCOR* (orange) to centromeric 3′ *CCNB3* (larger red) (red-yellow signals fused together, arrows).

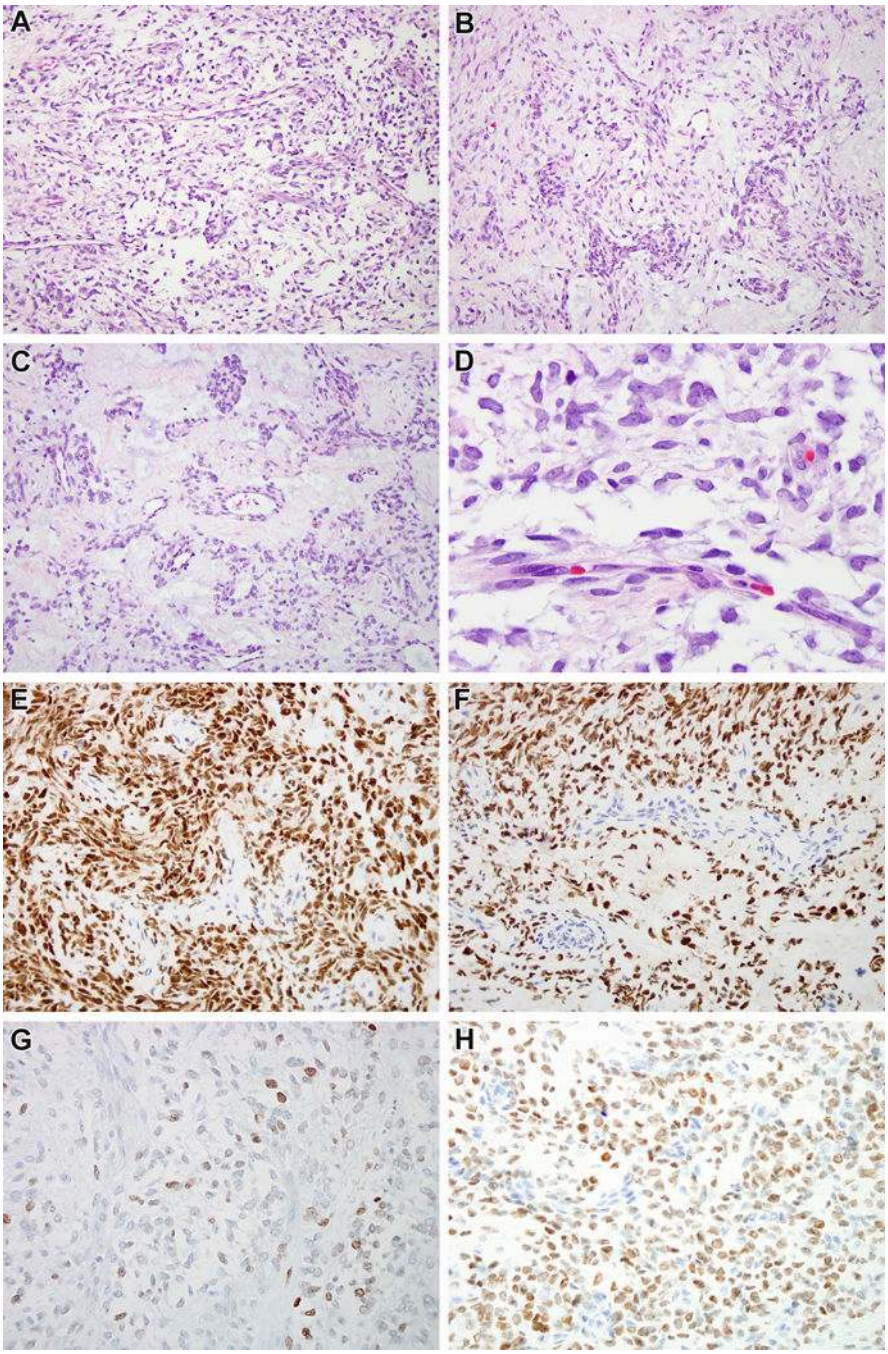

Figure 4.

BCOR-CCNB3 soft tissue sarcoma from comparison group. *BCOR-CCNB3* soft tissue sarcomas demonstrated morphologic features that overlap with CCSK, including a branching capillary vasculature pattern separating epithelioid to spindled cord cells that are set in a myxoid stroma (A). The cord cells appear to have clear cell cytoplasm, but in fact this represents extracellular matrix separating the cells (B). Stromal hyalinization mimics the sclerosing pattern of CCSK (C). The chromatin is fine and evenly dispersed similar to that of CCSK (D). Immunohistochemistry for BCOR highlights the neoplastic cord cells, with

absence of labeling in the septal cells (E, F), similar to that seen in CCSK. The cord cells demonstrate weak staining for PAX8 (G) and strong staining for SATB2 (H).

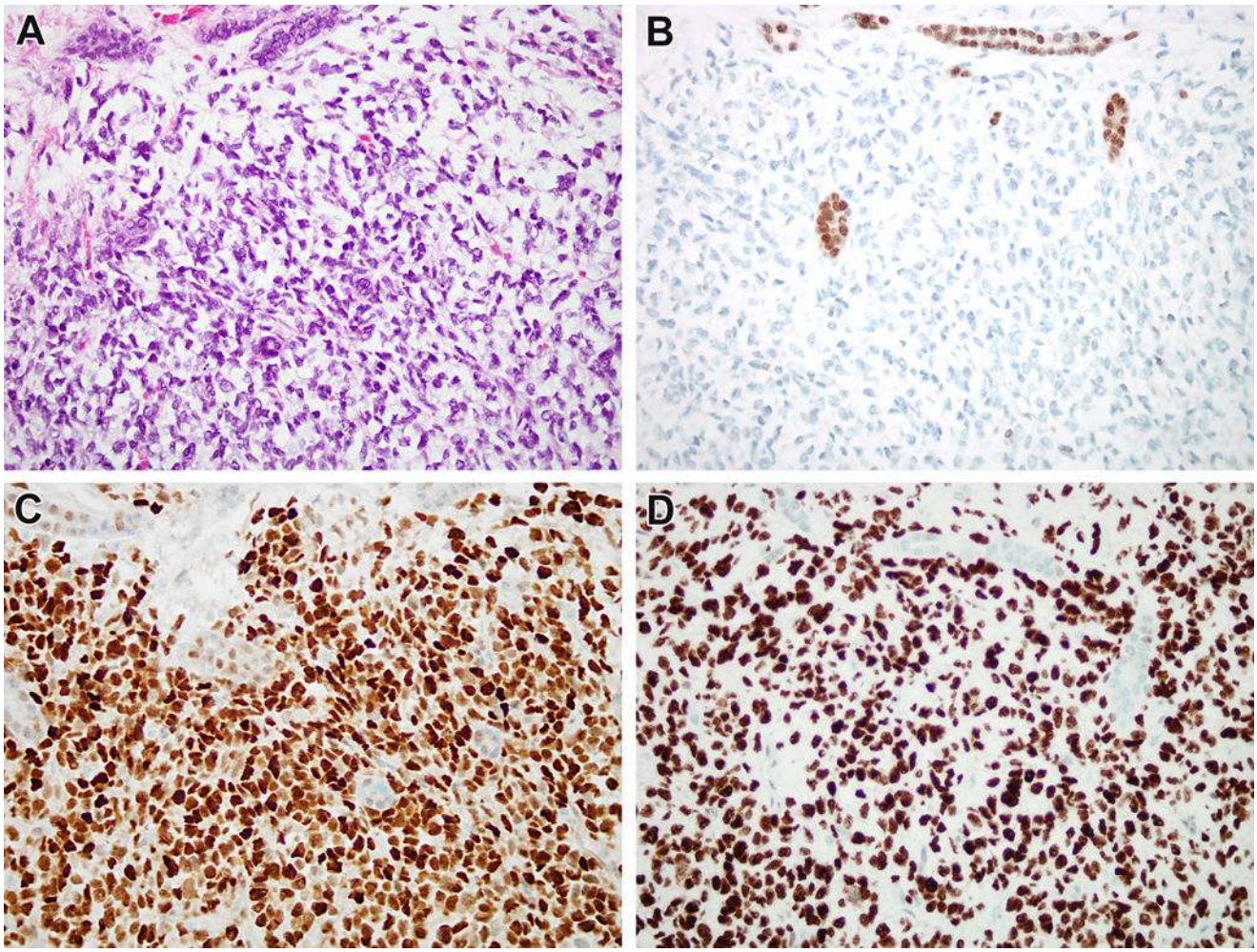

Figure 5.

Immunoprofile of CCSK. A. This CCSK from the control group demonstrates myxoid extracellular material separating cord cells, and branching capillary vasculature. Note the entrapped renal tubules at the upper and middle portions of the image. B. Like most cases, this CCSK was negative for PAX8. Note the entrapped native renal tubules serving as an internal control. C. The CCSK shows strong diffuse nuclear labeling for cyclin D1, while there is minimal weak labeling of the entrapped renal tubules. D. The neoplasm demonstrates strong diffuse nuclear labeling for TLE1.

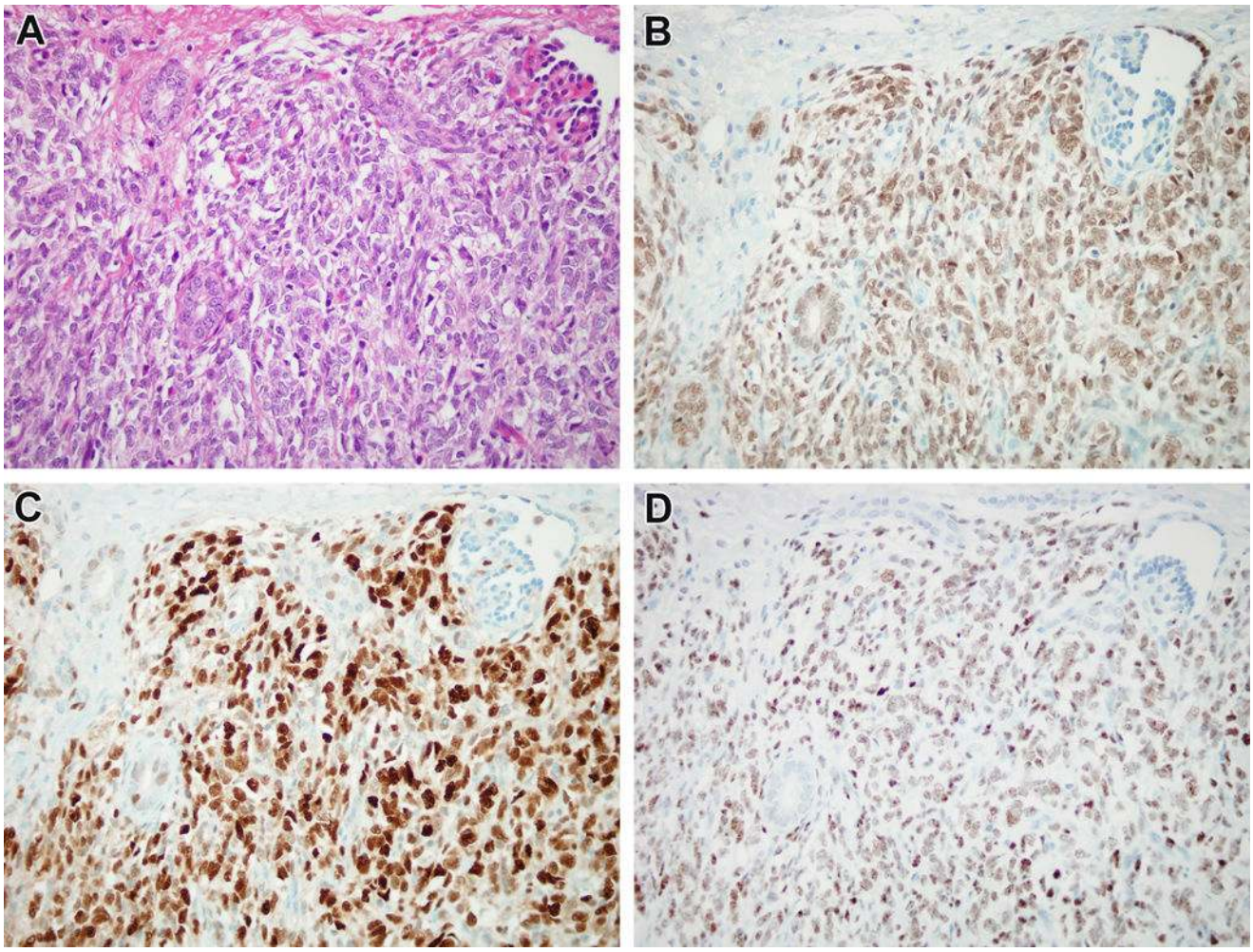

Figure 6.

Immunoprofile of CCSK. A. This typical CCSK from the control group demonstrates bland epithelioid cord cells separated by a branching capillary vasculature. Note the entrapped glomerulus at the upper right, and the entrapped native renal tubules to the left and center of the field. B. In this case, the neoplastic cord cells and native renal tubules show strong nuclear labeling for PAX8. C. The neoplastic cord cells demonstrate strong, diffuse nuclear labeling for cyclin D1, while there is minimal labeling of entrapped nephrons. D. The neoplasm demonstrates diffuse nuclear labeling for TLE1.

Table 1

Comparison of *BCOR*-related Neoplasms and Renal Synovial Sarcoma

	Typical CCSK	***BCOR-CCNB3* Renal Sarcomas**	**URCS/PMMTI**	***BCOR-CCNB3* Bone/Soft Tissue Sarcoma**	**Renal Synovial Sarcoma**
Usual Age	3 years (mean)	11.5 years (mean)	<1 year	Teens	43 years (median)
Morphology	Round/spindle cells separated by branching capillary vasculature in myxoid stroma; numerous variant patterns	Variably cellular spindle cell sarcoma associated with entrapped dilated renal tubules	Round to spindled primitive cells in myxoid background with	Cellular round/spindle cell sarcoma	Cellular spindle/round cell sarcoma often associated with entrapped dilated renal tubules
Genetics	*BCOR* ITD (90%); *YWHAE-NUTM2B/E* fusion (<10%)	*BCOR-CCNB3* fusion	*BCOR* ITD	*BCOR-CCNB3* fusion	*SS18-SSX* fusion
PAX8	20% positive	50% focal positive	NA	50% positive *****	Usually negative ****
BCOR	Positive **	Positive	Positive	Positive	50% positive
Cyclin D1	Positive ***	Positive	Positive	Positive	60% positive (weak)
SATB2	42% positive **	Positive	75% positive **	100% positive **	Minimal/negative
TLE1	80% positive	Positive	NA	Positive *	Positive

* data from reference 30. 2/2 *BCOR-CCNB3* soft tissue sarcomas in the current study were positive for TLE1

** includes data from reference 5

*** includes data from references 3 and 4

**** data from reference 31

***** data from reference 26. 1/1 *BCOR-CCNB3* soft tissue sarcoma in the current study was focally positive for PAX8

NA-not available

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Table 2

BCOR-Alteration Family of Neoplasms

Clear Cell Sarcoma of Kidney (CCSK)
BCOR-CCNB3 Sarcomas of Kidney and Bone/Soft Tissue
Undifferentiated Round Cell Sarcoma/Primitive Myxoid Mesenchymal Tumor of Infancy (URCS/PMMTI)