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Langerhans Cell Histiocytosis Associated With Renal Cell Carcinoma Is a Neoplastic Process

Clinicopathologic and Molecular Study of 7 Cases

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Abstract: Langerhans cell histiocytosis (LCH) is a rare histiocytic disorder composed of Langerhans cells admixed with reactive mononuclear and granulocytic cells, associated with prominent eosinophils. LCH is considered a neoplasm, driven in most cases by oncogenic \( \text{RAS}/\text{RAF}/\text{MEK}/\text{ERK} \) pathway mutations. The disease predominantly affects children. Urinary system involvement has rarely been reported in a multisystem disease setting. We describe 7 patients who presented with LCH occurring within (6 cases) or after (1 case) a resected clear cell (\( n=6 \)) or clear cell papillary (\( n=1 \)) renal cell carcinoma (RCC), identified prospectively in our routine and consultation files (2012 to 2019). The patients included 5 women and 2 men, with a median age of 54 years (range, 39 to 73 y), none with a history of LCH or LCH manifestations before the time of RCC diagnosis. The median size of the RCC was 3.5 cm (range, 1.8 to 8.3 cm). Treatment included partial (5 cases), or radical (2 cases) nephrectomy. All RCCs on gross examination showed at least focal cystic changes and were low grade (World Health Organization [WHO]/International Society of Urologic Pathologists [ISUP] grade 1 to 2). The LCH foci were detected as incidental histological finding within the resected RCC in all six cases and they were limited to few high-power fields (<2 mm\(^2\)) in 5 of 6 cases, but in the sixth case, they occupied almost the entire clear cell papillary RCC (2 cm nodule). No LCH manifestations were detected in the normal kidney or in perinephric fat. The seventh patient developed LCH within inguinal deep soft tissue followed by systemic manifestations 6 years after clear cell RCC. Langerhans cell immunophenotype was supported by the reactivity for S-100, CD1a, and langerin and by the negative pankeratin. Successful pyrosequencing of microdissected LCH DNA revealed the V600E \( \text{BRAF} \) mutation in all 6 cases of LCH within RCC. To our knowledge, only 3 similar cases were published since 1980; the only case tested for \( \text{BRAF} \) mutation showed wild-type \( \text{BRAF} \). This is the first study analyzing the morphologic and genetic features of a cohort of LCH associated with RCC. In our experience, these cases may be underrecognized in practice, or may erroneously be diagnosed as RCC dedifferentiation or high-grade sarcomatoid transformation. Finally, the detection of \( \text{BRAF} \) mutation further confirms that LCH in this setting is indeed a neoplasm, rather than a reactive lesion.

Key Words: renal cell carcinoma, RCC, Langerhans cell histiocytosis, kidney, concurrent, \( \text{BRAF} \) V600E

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Langerhans cell histiocytosis (LCH) is an uncommon proliferative histiocytic disorder composed of histologically bland Langerhans cells admixed with reactive mononuclear and granulocytic cells, often accompanied by prominent eosinophils.\(^1\) Since its description >1 century ago, much controversy has accompanied the etiology, pathogenesis, terminology, classification, and the treatment of LCH. Currently, it has been widely accepted that LCH represents a neoplastic disease of the Langerhans cells, driven in most cases by oncogenic mutations in the \( \text{RAS}/\text{RAF}/\text{MEK}/\text{ERK} \) pathway.\(^1–5\) Clinically, LCH may present as multifocal (eosinophilic granuloma), multifocal unisystemic (Hand-Schüller-Christian), or multifocal and multisystemic (Letterer-Siwe) disease, with variable clinical manifestations depending on the anatomic sites involved.\(^1,2\) The neoplastic hallmark cell of LCH, “the Langerhans cell,” is characterized by its histopathologic features, supplemented by the immunexpression for CD1a, langerin (CD207), and S-100
protein, and/or demonstration of Birbeck granules by electron microscopy. Both the localized and systemic diseases are predominantly affect children, whereas young adults are less frequently, and older people are very rarely affected. The treatment and the prognosis depend on the extent and dynamic of the disease.

Systemic LCH may involve visceral organs, such as lung and liver, in addition to bone, bone marrow, lymph nodes, and spleen. Urogenital involvement by LCH is very rare and has been typically documented in patients with the multisystemic disease.

In this study, we describe a cohort of 7 LCH lesions associated with renal cell carcinoma (RCC), encountered in our routine and consultation practice, and all but 1 case were identified incidentally within resected RCCs. This finding was a source of confusion and created diagnostic uncertainty that led to consider in the differential diagnosis, either RCC de-differentiation or high-grade sarcomatoid transformation. Review of the literature uncovered only 3 similar cases, published as individual case reports since 1980 (Table 1, cases 1 to 3), with only 1 reported in a pathology journal. We, therefore, propose that this phenomenon is possibly more frequent than the literature implies and potentially significantly underrecognized by pathologists. We hope that our report will draw the attention of general surgical and urological pathologists to this potentially misleading finding.

### MATERIALS AND METHODS

The cases have been identified by the authors during the period from 2012 to 2019. Only case 7 predates this period and was identified by the first author. No retrospective search for additional cases in our archives was performed. The LCH was detected as incidental histologic finding within the resected RCC in 6 cases and developed years after nephrectomy for clear cell renal cell carcinoma (ccRCC) in 1 patient. Diagnosis and subtyping of the RCC have been reviewed according to the current World Health Organization (WHO) classification. LCH was diagnosed in accordance with established histopathologic and immunophenotypic criteria.

All samples have been formalin-fixed and processed routinely for histopathologic evaluation. Gross reports were evaluated for size, location, and laterality of the tumor and for the presence of cystic features on gross examination.
A cystic component was also recorded if present on microscopic evaluation. Immunohistochemistry (IHC) was performed on 3µm sections cut from paraffin blocks using a fully automated system (“Benchmark XT System,” Ventana Medical Systems Inc., Tucson, AZ) and the following antibodies: S-100 protein (polyclonal, 1:2500; Dako), CD1a (clone EP81, 1:100; Quartett/Epitomics), cyclin D1 (clone SP4, 1:50; Zytomed), langerin/CD207 (clone 12D6, 1:100; Novocastra), pankeratin (clone AE1/AE3, 1:40; Zytomed), CK7 (OV-TL, 1:1000; Biogenex), and PAX8 (rabbit polyclonal, 1:50; Cell Marque). The BRAF IHC was performed using the VE1–mutation-specific mouse monoclonal antibody (clone ab228461, 1:100; Abcam). Only cytoplasmic staining was considered specific.

**BRAF Mutation Testing**

**Microdissection and DNA Isolation**

For molecular analysis, 5µm sections were cut from formalin-fixed paraffin-embedded tissue specimens, deparaffinized, and rehydrated. The LCH foci were microdissected manually with a sterile scalpel. DNA isolation was performed using the Maxwell 16 system and the Maxwell 16 LEV Blood DNA Kit (Promega, Madison, WI) according to the manufacturer’s instructions. Analysis of BRAF V600 mutation hotspot was performed at the institutional-accredited molecular diagnostics laboratory using standardized and validated in-house protocols (including DNA from the colon carcinoma cell line HCT116 as a BRAF p. V600 wild-type control, DNA from the colon carcinoma cell line HT 29 as a BRAF p. V600E positive control as well as a reaction mix without DNA as a negative control) and a PyroMark Q24 system (QIAGEN, Hilden, Germany) according to manufacturer’s instructions.

**RESULTS**

**LCH Detected Within Resected RCC (n = 6)**

**Clinical Features**

The clinicopathologic features are summarized in Table 1 (cases 4 to 9). Six patients who had LCH within a resected RCC were identified. The 6 patients include 4 women and 2 men aged 39 to 73 years (median, 54 y). None had a prior history of LCH, and diagnosis of LCH was first made after the evaluation of the nephrectomy specimens, because the LCH lesions were confined to the RCC tissue, and represented incidental histologic findings in all cases. Additional clinical investigations showed no evidence of systemic disease in any of the patients. Of note, 1 patient had numerous basal lung nodules consistent with a reactive process, but biopsies were not obtained and the possibility of pulmonary LCH cannot be ruled out with certainty. All tumors but one were located in the left kidney. The treatment included partial (5/6) and radical (1/6) nephrectomy. Follow-up was available for 5 patients and ranged 6 to 60 months (median, 17 mo). All patients remained RCC free at the last follow-up, and none had systemic LCH manifestation, neither in the kidney nor in other organs.

**Pathologic Findings**

Five patients had solitary RCC ranging in size from 1.8 to 8.3 cm (median, 3 cm). The sixth patient had a multifocal (n = 4) RCC ranging in size from 0.4 to 3.5 cm. The LCH lesion in this patient was located within the largest RCC nodule. Grossly, the RCCs were well-circumscribed with variegated, variably cystic and solid, yellow-tan cut-surface, and focal hemorrhagic areas. Histologic examination was consistent with conventional ccRCC in 5 patients and with clear cell papillary renal cell carcinoma (ccPRCC) in 1 case. Notably, all RCCs showed at least focal cystic changes that were typically recognizable grossly. All RCCs had low-grade nuclear features according to the WHO/International Society of Urologic Pathologists (ISUP) grading (grade 1 to 2); none showed coagulative necrosis, rhabdoid, or sarcomatoid features. All RCCs were confined to the kidney, without evidence of vascular invasion, perinephric fat involvement, or other aggressive features.

Within the RCC itself, the LCH foci presented either as basophilic staining cellular foci, sharply demarcated from the surrounding RCC tissue or as multifocal hemorrhagic foci (Figs. 1A–D). At higher magnification, atypical histiocytic infiltrates showing cells with convoluted nuclear contours and nuclear grooves were appreciated (Fig. 1E). The cells had moderate pale to eosinophilic cytoplasm, compatible with the histologic features of Langerhans cells. Numerous eosinophils were also present in the background (Fig. 1E). In other areas showing paucity of eosinophils and predominance of Langerhans cells, the abrupt transition from the RCC to the basophilic staining LCH tissue mimicked high-grade transformation (Fig. 1F). The LCH foci were in most cases limited to only a few high-power microscopic fields. However, in 1 case (ccPRCC), the LCH areas occupied almost the whole RCC nodule, with only minimal ccPRCC. The ccPRCC in this case was hard to identify on hematoxylin and eosin staining in most of the slides (Figs. 2A–F), but was readily highlighted by IHC stains with CK7, PAX8, and CA-9. The LCH showed variable cellularity and eosinophils (Figs. 2B, C). Immunohistochemically, the LCH cells were uniformly positive for langerin (Fig. 3A), CD1a (Figs. 3B, C), S-100 (Fig. 3D), and cyclin D1 (Fig. 3E) and were negative for pancytokeratin AE1/AE3 and PAX8. The ccPRCC strongly expressed CK7 (Fig. 3F). The cystic spaces were predominantly lined by LCH cells (Figs. 3A, B) and focally by RCC cells (Fig. 3C).

**Molecular Findings**

The BRAF V600 analysis gave interpretable results in all 6 cases, and positive and negative controls showed the expected results underlining the validity of the analyses. The analysis revealed the c.1799T > A transversion in all cases, indicating the classic BRAF V600E mutation. IHC with the VE1–mutation-specific antibody was successful in 3 cases (cases 6, 7, and 9); all showed homogenous cytoplasmic reactivity limited to the neoplastic LCH cells (Fig. 4). Surrounding RCC cells are negative.
This concerns a 58-year-old female patient who had a history of left radical nephrectomy performed for a 6 cm ccRCC originating in the upper pole of the left kidney. She presented 6 years later with a 13-cm inguinal deep soft tissue mass that was resected (Fig. 5A). Histology showed mass-forming LCH with the involvement of adjacent lymph nodes as well (Fig. 5B). Features of frankly malignant Langerhans cell sarcoma were absent. Over the next 9 years, the patient presented with multiple recurrences of LCH with progressive multifocal gluteal, pleura, and mediastinal LCH manifestations. Attempted molecular testing of this case was unsuccessful owing to poor DNA quality. The ccRCC slides from 1998 were retrieved and reviewed. Histology showed conventional ccRCC (ISUP/WHO grade 2) with variable cystic foci. There was no angioinvasion, perirenal involvement, or other aggressive features. No LCH was detected within the RCC. There was, however, only 4 paraffin blocks available from the 6 cm large RCC, and extensive sampling was not done.

**FIGURE 1.** Whole mount sections showing distribution and extent of LCH within ccRCC (A, hematoxylin and eosin; B, an overview of langerin immunostaining highlighting the LCH). C, ccRCC with solid and cystic areas associated with multiple hemorrhagic LCH foci. D, Eosinophil-rich LCH infiltrate associated with fibrosis might be mistaken for regressive changes. E, Higher magnification of the LCH shows prominent eosinophils and characteristic cytology of LCH cells. F, This eosinophil-poor LCH focus closely mimics the high-grade transformation of the RCC. Images (A) and (B) from case 8; C–F from case 6 (for case numbers see Table 1). Copyright © 2018 Michael Bonert, MD, FRCP. You are free to share and adapt these image as per the CC BY-SA 4.0 (https://creativecommons.org/licenses/by-sa/4.0/legalcode; https://commons.wikimedia.org/wiki/File:Langerhans_cell_hистiocytosis_within_RCC_-_extremely_low_mag.jpg; https://commons.wikimedia.org/wiki/File:Langerhans_cell_hистiocytosis_within_RCC_-_low_mag.jpg; https://commons.wikimedia.org/wiki/File:Langerhans_cell_hистiocytosis_within_RCC_-_very_high_mag.jpg).

**LCH Following RCC (n = 1)**

This concerns a 58-year-old female patient who had a history of left radical nephrectomy performed for a 6 cm ccRCC originating in the upper pole of the left kidney. She presented 6 years later with a 13-cm inguinal deep soft tissue mass that was resected (Fig. 5A). Histology showed mass-forming LCH with the involvement of adjacent lymph nodes as well (Fig. 5B). Features of frankly malignant Langerhans cell sarcoma were absent. Over the next 9 years, the patient presented with multiple recurrences of the LCH with progressive multifocal gluteal, pleura, and mediastinal LCH manifestations. Attempted molecular testing of this case was unsuccessful owing to poor DNA quality. The ccRCC slides from 1998 were retrieved and reviewed. Histology showed conventional ccRCC (ISUP/WHO grade 2) with variable cystic foci. There was no angioinvasion, perirenal involvement, or other aggressive features. No LCH was detected within the RCC. There was, however, only 4 paraffin blocks available from the 6 cm large RCC, and extensive sampling was not done.
DISCUSSION

The pathogenesis of LCH has puzzled scientists and clinicians for decades since the entity was recognized last century. The dual theory “reactive-inflammatory versus neoplastic” has evolved into the favor of the neoplastic hypothesis based on demonstration of clonality of the LCH cells and a high incidence of somatic activating \textit{BRAF} V600E point mutation.\textsuperscript{3–5} The frequency of \textit{BRAF} V600E mutation in LCH seems to vary based on the method used for analysis and was reported to be as high as 70% in some series.\textsuperscript{1,3–5} Furthermore, \textit{MAP2K1} mutations were detected as well, mainly in \textit{BRAF} wild-type, and also in a small subset of \textit{BRAF} mutant lesions.\textsuperscript{3–5} Besides \textit{BRAF}, some lesions harbored additional deleterious \textit{TP53} mutations.\textsuperscript{5} Likewise, expression of cyclin D1 was observed in neoplastic LCH but not in clearly reactive lesions.\textsuperscript{12} All these observations are consistent with a true neoplastic lesion and argue against the old reactive-inflammatory theory.

LCH has been reported to be associated with a variety of hematological and solid malignancies, of which lymphoma, leukemia, and lung carcinoma are the most common.\textsuperscript{13–15} LCH may occur before, after, or be concomitant with a separate malignancy. Among solid tumors associated with LCH, carcinoma of the lung and the thyroid gland are the major types encountered, followed by rare cases of breast cancer, liver cancer, sarcomas, and central nervous system tumors.\textsuperscript{13–15} Diagnosis of the
carcinoma and the LCH was concurrent in most lung carcinoma and thyroid carcinoma cases. Notably, the LCH was confined to the lung in cases of lung carcinoma. Moreover, the LCH lesions were intimately associated with the carcinoma or lymphoma tissue in most cases, so that both entities usually were diagnosed concurrently as in our cases. Hammar et al detected Langerhans cells in close association with the neoplastic epithelial cells in 7 of 37 bronchoalveolar lung carcinomas. These observations, which are very similar to our current study, suggested that the LCH lesions in these cases represent a dendritic cell reaction in response to a stimulus related to the associated solid cancer (lung carcinoma or lymphoma). On the contrary, most solid cancers preceded by LCH developed at the site of irradiation for the LCH, indicating that the association between solid cancer and the LCH is coincidental and is likely a consequence of irradiation.

The reported coexistence of LCH and RCC, as in our current series, is extremely rare. The frequency of LCH detected within a resected RCC is unknown. In our institutional files, RCCs containing foci of LCH represent 0.1% to 0.3% of all RCC cases diagnosed during the study period. There are only 3 previous reports in the English literature from 1980, 2011, and 2018 with the more recent report published in a surgical pathology journal. The first previously reported case was that of a 46-year-old man with a history of eosinophilic granuloma of the left mastoid at age 12, treated with curttage and radiation. Thirty-four years after the initial presentation, the patient was found to have an eosinophilic granuloma of the left mid-humerus, in addition to a 1 cm

**FIGURE 3.** IHC revealed diffuse expression of langerin (A), CD1a (B, C), S-100 (D), and cyclin D1 (E). Note the lining of cystic spaces by LCH cells in (A) and (B) and by alternating CD1a-positive LCH cells (lower part, red arrows) and CD1a-negative RCC cells (upper part, black arrows). F. The ccPRCC case (left part of the image) shows homogeneous CK7 reactivity, while the LCH on right is negative. Images A, B, D, E, and F from case 7. Image C from case 5.
RCC in close association with LCH in the right kidney.8 The second and third previously reported cases were of a 60-year-old woman and a 60-year-old man who were incidentally found to have a renal mass, which was histologically confirmed to be RCC, harboring microscopic foci of LCH.9,10 In all 3 cases, the LCH was intimately associated with the RCC component. Six of the 7 cases included in the current study demonstrated this same feature.

The fundamental pathogenesis of the association of LCH with other tumors is largely unknown. Most authors speculate that the tumor microenvironment may play a critical role in recruiting Langerhans cells to migrate and proliferate in organs/tissues where tumor cells reside, as they can produce a wide variety of cytokine/chemokine-like factors with mitogenic and chemotactic functions.8,13 In our cases, the findings of LCH in close association with the RCC, and without other manifestations of systemic LCH disease, imply that a focal LCH lesion occurred as a secondary reaction to the primary tumor, the RCC.13 The pathogenesis of this peculiar phenomenon is unclear.

Previous studies on LCH associated with diverse malignant neoplasms suggested both a reactive etiology (especially for those lesions intimately associated with a synchronous malignancy such as malignant lymphoma and lung adenocarcinoma),13 as well as therapy-related pathogenesis (for those cases with a remote history of a previous malignancy such as leukemia and cases where solid cancer developed in the irradiation field for the LCH).13 To our knowledge, only one of the previously reported LCH cases within RCC was investigated for BRAF mutations and showed negative results.10 Accordingly, reactive pathogenesis remained likely.

We, therefore, examined all cases for BRAF mutations trying to address the question of reactive LCH lesions versus genuine neoplastic LCH. Indeed, all of 6 analyzable cases revealed the same V600E BRAF mutation, thus confirming that these represented true neoplastic LCH and excluding an LCH-like reactive-inflammatory lesion in response to the RCC. However, the answer to the question of why the LCH did develop within the RCC proper itself remains elusive.

FIGURE 4. A, IHC using the VE1–mutation-specific antibody highlights specifically the neoplastic LCH cells including those LCH cells lining the cysts (note absence of staining in lymphoid cells in B). Images from case 7.

FIGURE 5. This 13-cm deep soft tissue mass (A) excised from the inguinal area of a 58-year-old woman with a history of ccRCC proved to be a huge mass-forming LCH (B). A review of the slides of the previous ccRCC showed no LCH. Images from case 10.
Likewise, the observation that all cases and likely all previously reported cases (except for 1 ccPRCC) are low-grade ccRCC with variable cystic changes remains unclear. The prevalence of ccRCC in this unique small cohort is possibly not by chance; this suggests the subtype may play a role in the coexistence of these 2 distinct entities, but the low number of cases does not allow for statistical analysis. All 6 cases described herein did not show any progression or wide dissemination of the LCH after resection of the RCC. This supports an indolent nature of the LCH and that resection of the “host” RCC was a sufficient treatment for both lesions, although close clinical follow-up is warranted. The seventh additional case we describe herein showed progressive systemic LCH developing years after ccRCC in a 58-year-old female patient. Although we were not able to clearly identify LCH lesions within the ccRCC in this patient, it cannot be completely ruled out that this patient’s ccRCC did harbor foci of LCH that were possibly not sampled, given that only limited number of slides were provided from her large RCC.

From a diagnostic viewpoint, the most relevant message would be to distinguish LCH within RCC from focal high-grade sarcomatoid transformation or dedifferentiation of the RCC. Indeed, one of the cases was sent to us with a diagnosis of dedifferentiated RCC. Therefore, a careful assessment of the morphology is required for distinguishing LCH cells from the RCC cells. However, a subset of RCC of different types (chromophobe carcinomas in particular) may rarely display prominent nuclear grooves, resulting in coffee bean-shaped nuclei that may resemble those of LCH. However, these cells are usually present throughout the neoplasm and are not associated with an eosinophil-rich inflammatory background as seen in LCH lesions. IHC provides a reliable way to distinguish LCH (positive for S-100, CD1a, and langerin) from grooved RCC cells (positive for cytokeratins and PAX8). Another possibility to be considered when encountering a strange-looking cell population within an otherwise low-grade RCC is “tumor-to-tumor metastasis.” Indeed, RCC represents the most common recipient of metastases, the donor neoplasm being lung carcinoma in the majority of cases. Up to 15% of cases of RCC with a concurrent second malignancy harbored metastatic foci. Last but not least, the detection of a BRAF mutation in such RCC specimens with foci of LCH should not be mistaken for BRAF-mutated RCC with the consequence of false therapeutic recommendations. Although being universally present in metanephric adenomas, BRAF mutations seem to be very rare in ccRCC. The mutation-specific anti-BRAF VE1 antibody confirmed the presence of the mutated BRAF protein in the LCH cells and its absence in the surrounding RCC cells.

In summary, we describe 6 cases of low-grade RCC harboring foci of LCH located within, and in 1 case replacing the RCC to a variable extent, and the seventh case of progressive LCH developing years after ccRCC. LCH within resected RCC may be mistaken for high-grade sarcomatoid transformation if not correctly identified. The presence of BRAF mutations in all intra-RCC LCH lesions confirmed a true neoplasm (tumor-in-tumor development) and argued against a reactive process. The pathogenesis of this rare finding remains to be explored in future studies.

REFERENCES