Unique Case of a Rare Mesenchymal Tumor Harboring a Somatic c.119delC VHL Mutation

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INTRODUCTION

The VHL gene is a tumor suppressor gene located at chromosome 3p25, and its protein has multiple functions linked to multiple effector proteins. Aberations in the VHL gene are associated with sporadic clear cell renal cell carcinomas (ccRCCs), sporadic hemangioblastomas, pheochromocytomas, pancreatic islet cell tumors, endolymphatic sac tumors, and benign cysts affecting various organs. Germline inactivation of the VHL gene causes the autosomal dominant von Hippel-Lindau (VHL) syndrome. VHL is rarely mutated outside of the context of RCC or VHL syndrome–associated malignancies. Biallelic VHL inactivation caused by genetic and epigenetic alterations (including DNA methylation, histone modifications, and coincidental loss of genes localized adjacent to the VHL chromosome locus) has been described. In both hereditary and sporadic tumors, VHL mutations are heterogeneous.

The encoded VHL protein (pVHL) plays an important role in ubiquitination and proteasomal degradation of hypoxia inducible factor-1 α (HIF-1α). HIF-1α activates transcription of genes (ie, target genes related to adaptation to hypoxia, such as vascular endothelial growth factor [VEGF] and platelet-derived growth factor β [PDGFβ]) and acts in cellular processes such as metabolism, cellular senescence, chemotaxis, proliferation, transcription, WNT signaling, ubiquitinating RNA polymerase, and regulating nuclear factor κB. Here, we present a case report of a patient with metastasized follicular dendritic cell sarcoma (FDCS) harboring a somatic c.119delC VHL mutation. Our aim was to investigate the (epi)genetic background of this patient’s disease and to determine whether the identified VHL mutation could be a driving mutation in this patient’s FDCS.

METHODS

Case Report

In 2013, a 29-year-old female patient presented with a large abdominally located mass and multiple hepatic lesions. A histologic biopsy from a liver lesion showed an epithelioid and spindle cell malignant neoplasm with scattered lymphocytes. Immunohistochemistry (IHC) results matched the pattern of FDCS with expression of follicular dendritic cell markers CD23, and CD35 (Fig 1A-C). Ewing sarcoma breakpoint region 1 (ESWR 1) was not detected with fluorescence in situ hybridization. On the basis of radiologic diagnosis, the pattern did not fit an RCC. This was further supported by a negative paired box gene 8 (PAX8) IHC result (Fig 1D).

The clinical course of our patient’s disease is outlined in Figure 2. The first-line treatment consisted of eight cycles of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), resulting in a metabolic complete response. Approximately 8 months later, progressive disease was apparent, with fluorodeoxyglucose-positive lymph nodes in the hepatic hilum (Fig 3A and 3B). The second-line treatment was pazopanib, which resulted in unexpected stable disease for 22 months.

The patient participated in the Dutch National Center for Personalized Cancer Treatment (CPCT-02) program, rendering additional tumor sampling for whole-genome sequencing (WGS) analysis before and after treatment with pazopanib. DNA was extracted from fresh-frozen biopsies obtained from a hepatic metastatic lesion and blood as a germline control. WGS was performed on the Illumina HiSeq X platform with 100 ng DNA as input using standard protocols (paired-end 2 × 150 base pairs; Illumina, San Diego, CA). Within the framework of CPCT-02, all germline variants were filtered, which guarantees that only somatic variants are reported. Tumor samples and control blood were sequenced with a minimum base coverage depth of 90x and 30x, respectively. Somatic single nucleotide and indel variant calling was performed by an optimized bioinformatics pipeline (https://github.com/hartwigmedical/) on the basis of Strelka2 (v1.0.14-1). Additional filtering against a panel of nearly 2,000 control genomes removed variants that were present in six or more of these respective samples. Germline and somatic structural variant detection was performed using Illumina Manta (v.1.0.3) and were post-processed using a custom application (Break Point Inspector; https://github.com/hartwigmedical/) to filter false-positive candidates and to discover exact breakpoint positions. In both biopsies, WGS showed a somatic c.119delC VHL mutation, which prompted us to identify the meaning and importance of this variant in our patient.
RESULTS

Genomic Landscape

The identified c.119delC VHL frameshift mutation led to a truncated pVHL (p.Pro40fs; Fig 4). Both biopsies showed genome-wide aberrations, with large chromosomal copy number alterations, structural rearrangements, and mutations in a broad spectrum of loci (Fig 5). Amplification of the VHL locus 3p25 was identified in both biopsies, with germline-informative B-allele frequencies revealing loss of heterozygosity. This finding hints toward a model in which the remaining VHL allele had been duplicated (and subsequently mutated), resulting in three VHL loci in biopsy 1 (two VHL<sub>WT</sub> and one VHL<sub>c.119delC</sub>) and seven VHL loci in biopsy 2 (two VHL<sub>WT</sub> and five VHL<sub>c.119delC</sub>). Identified variants and translocations differed between biopsies 1 and 2, with a remarkable reduction in translocations in the post-treatment biopsy (Fig 5).

Consequences of the Identified c.119delC VHL Mutation

The primary question about the identified VHL mutation concerned the functional consequence at the protein level and whether this variation could be a driver mutation. Overproduction of HIF-1α in the absence of hypoxia is the main effect of pVHL functional loss. Therefore, we performed IHC on the second biopsy for HIF-1α (Fig 1E),

![Image](image_url)

**FIG 1.** Immunohistochemistry (IHC). The liver biopsy showed large solid fields of discohesive epithelioid and spindle cells with basophilic cytoplasm, round to oval nucleus, and moderately enlarged nucleoli. A moderate number of scattered lymphocytes was present throughout the lesion. The cells were moderately pleomorphic with frequent mitoses. Hematoxylin and eosin immunohistochemistry (HE IH) ×100 (A). The tumor cells were strongly positive for CD21 (B), CD23 (C), and SMA; CD35 was locally positive. Calretinin, CD20, CD117, chromogranin, desmin, DOG1, EMA, KERPAN, KL1, Melan A, S100, and synaptophysin were negative. Paired box gene 8 (PAX8) IHC was negative (D). HIF-1α IHC showed positive nuclear staining (E), and glucose transporter 1 (GLUT1) IHC showed positive staining (F).

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<tr>
<th>Treatment</th>
<th>T0</th>
<th>Eight Cycles of CHOP</th>
<th>T6</th>
<th>No Treatment</th>
<th>T14&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Pazopanib</th>
<th>T36&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Phase 1 Trial</th>
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**FIG 2.** Clinical course of the disease. The patient was diagnosed with follicular dendritic cell sarcoma in November 2013 (T0). The patient received eight cycles of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), which resulted 6 months later (T6) in metabolic complete remission (CR). Eight months later (T14), the patient had progressive disease (PD). At T14, she was treated with pazopanib. In response to pazopanib, the patient had stable disease (SD) for a total of 22 months. After that (T36), the patient showed PD and participated in a phase 1 trial with immunotherapy (CD40 agonistic monoclonal antibody) in combination with vanucizumab (anti-angiopoietin-2 and anti-VEGF [vascular endothelial growth factor]) for 11 months. (*) Tumor biopsies were obtained before the start of treatment with pazopanib and after its discontinuation.
which showed positive nuclear staining, and for glucose transporter 1 (GLUT1; a glucose transporter associated with HIF-1α downstream activation), which showed positive staining (Fig 1F). Both results provide circumstantial evidence for functional loss of pVHL.7,10

DISCUSSION

Our analysis revealed a unique patient with FDCS harboring a somatic functional VHL aberration, which is the first description of a VHL mutation in sarcoma.14 Because FDCS is a rare mesenchymal neoplasm with a largely unknown and rather complex genetic landscape15 and is unknown for harboring VHL aberrations, we verified specific IHC-based markers to confirm the diagnosis and to reject metastatic RCC (mRCC) as an alternative diagnosis. The positive HIF-1α IHC result may indicate a functional loss of pVHL; in this patient, it was a consequence of mutation and potential methylation-derived changes leading to biallelic VHL gene inactivation. Although most evidence in FDCS has been found for the involvement of the RAS/RAF signaling pathway,16,17 we did not identify mitogen-activated protein kinase (MAPK) alterations in our patient. All evidence collected points toward FDCS with a functional VHL mutation. A mutant allele-specific imbalance as a consequence of allele-specific amplification has been described.18,19 However, this seems to be a more common aspect with activating mutations. Recurrent VHL locus amplifications have not been described in ccRCC; therefore, it does not seem to be a common aberration. However, these reports did not specifically investigate post-tyrosine kinase samples.2,20,21

Numerous nonspecific chromosomal translocations were present in the pretreatment biopsy, with a remarkable decline in the number of structural variants (SVs) following treatment with pazopanib. Treatment of cancer may have an influence on involved processes and subsequently on patterns and frequency of SVs.22 Alterations in the number of SVs between time points in a patient’s malignancy may also be a consequence of tumor evolution and selective survival of subclonal populations as a result of the selective pressure of treatment, similar to variable somatic alterations that cause the emergence of drug resistance.23 The changes in the genomic landscape between the two biopsies obtained in our patient could be associated with the observed disease response during treatment with pazopanib.24 Moreover, the fact that the mutated VHL gene has been amplified in our patient’s disease over time emphasizes its importance and potential as a driver mutation.

Upregulated VEGF is associated with the HIF pathway. In ccRCC, it is well known that HIF-1α is constitutively activated by inactivation of the VHL gene. Pazopanib, a multitargeted inhibitor with activity against the VEGF receptor and platelet-derived growth factor α and β receptors, is effective in the treatment of mRCC25 and metastatic sarcoma.26 During treatment with pazopanib, our patient had stable disease for 22 months. The length of this progression-free survival (PFS) is significantly longer than

FIG 3. Localization and progression of our patient’s follicular dendritic cell sarcoma. In March 2016, progressive disease was revealed on a conventional CT scan (A) and on a positron emission tomography scan (B) before the start of treatment with pazopanib. In January 2017, progressive disease persisted after 22 months of treatment with pazopanib (C).
the observed median PFS in the PALETTE trial (4.6 months; 95% CI, 3.7 to 4.8 months). Moreover, Saygin et al performed a pooled analysis of data from 462 patients with FDCS, showing a median survival for patients with metastatic disease of 9 months (range, 0.25 to 72 months) and a 2-year survival rate of 15.8%; these results suggest that the long PFS is not the result of better prognostic features of FDCS. The relatively long PFS during our patient’s last treatment, which consisted of the combination of a CD40 agonistic monoclonal antibody and an anti-angiopoietin-2 and anti-VEGF bispecific monoclonal antibody, could possibly be explained by the effect of the latter drug (vanucizumab). These data imply that the VHL mutation in our patient may predict a biologic behavior more similar to mRCC than to a metastatic sarcoma in response to treatment with pazopanib. These findings are not sufficient to make an argument for VEGF-targeted therapies in FDCS, because this is the first case of a VHL-mutated FDCS as far as we know.

Although HIF stabilization and GLUT1 accumulation are, at the most, indirect evidence for pVHL functional loss, a limitation of this case report is the absence of methylation analysis looking further into epigenetic silencing of the VHL gene. This is a consequence of the lack of normal control material from our patient, which made it impossible to correctly interpret the methylation analysis. In addition, there was no remaining material from biopsy 1 to perform additional IHC analysis.

In conclusion, this case report describes a patient with FDCS harboring a unique somatic mutation in VHL. This case underscores the scientific value of next-generation sequencing of a patient’s genetic material to unravel the genomic profile of cancers and to identify potential genetic abnormalities that can be targeted by cancer therapies.

FIG 4. Change-of-function von Hippel Lindau (VHL) frameshift mutation (c.119delC) leads to truncated VHL protein (pVHL). (A) c.119delC mutation on a schematic model of premRNA VHL transcript with allelic frequencies of reference (GC) and alternative (G-) observations, colored white and gray, respectively. Untranslated regions (UTR) and exonic regions are depicted by rectangular boxes (colored dark blue and dark gold, respectively), and intronic regions are depicted by black lines. (B) c.119delC mutation on a schematic model of pVHL with predicted protein domains; (GXEEEX)8 is shown in violet, α-domain in purple, and β-domain in pink. (C) Protein sequences of wild-type (wt) pVHL and mutant pVHL, colored by protein domains; (GXEEEx)8 is shown in light blue, α-domain in light gold, and β-domain in red. (The VHL gene model is on the basis of Ensembl transcript NM_000551.2 and the protein model is on the basis of the UniProtKB P40337 entry.) Red asterisk indicates stop codon. Bold text represents (predicted) aberrant sequence of amino acids (out-of-frame). BAF, B-allele frequencies.

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**FIG 5.** Genomic landscapes before and after treatment. (A) Biopsy obtained before pazopanib treatment. (B) Biopsy obtained after pazopanib treatment. The outermost track displays the ideogram of the human genome with exclusion of chromosome Y. The second outermost track displays absolute copy number estimations from zero copies to 10 copies. Regions with copy number gains (more than three copies; green) and copy number losses (zero copies; red) are shown. The third track displays the B-allele frequencies (BAFs) in the tumor(s) with germline-informative (heterozygous) markers. High numbers, indicated in blue, represent regions with more mutations. The innermost lines represent structural variations and interchromosomal translocations are shown in blue, deletions in pink, insertions in green-cyan, inversions in yellow-brown, and tandem duplications in brown.
REFERENCES


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