Adrenocortical carcinoma and succinate dehydrogenase gene mutations: an observational case series

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Abstract

Objective: Germline loss-of-function mutations in succinate dehydrogenase (SDHx) genes results in rare tumor syndromes that include pheochromocytoma, paraganglioma, and others. Here we report a case series of patients with adrenocortical carcinoma (ACC) that harbor SDHx mutations.

Patients and results: We report four unrelated patients with ACC and SDHx mutations. All cases presented with Cushing syndrome and large adrenal masses that were confirmed to be ACC on pathology. All four ACC specimens were found to have truncating mutations in either SDHC or SDHA, while cases 1, 2 and 3 also had the mutations confirmed in the germline: Case 1: SDHC c.397C>T, p.R133X; Case 2: SDHC c.43C>T, p.R15X; Case 3: SDHA c.91C>T, p.R31X; Case 4: SDHA c.1258C>T, p.Q420X. Notably, Case 1 had a father and daughter who both harbored the same SDHC germline mutation, and the father had a paraganglioma and renal cell carcinoma. A combination of next generation sequencing, and/or immunohistochemistry, and/or mass spectroscopy was used to determine whether there was loss of heterozygosity and/or loss of SDH protein expression or function within the ACC. Potential evidence of loss of heterozygosity was observed only in Case 2.

Conclusions: We observed truncating mutations in SDHA or SDHC in the ACC and/or germline of four unrelated patients. Given how statistically improbable the concurrence of ACC and pathogenic germline SDHx mutations is expected to be, these observations raise the question whether ACC may be a rare manifestation of SDHx mutation syndromes. Further studies are needed to investigate the possible role of SDH deficiency in ACC pathogenesis.

Introduction

Adrenocortical carcinoma (ACC) is a rare malignancy of the adrenal cortex (estimated prevalence: 1 per 2 million) (1) with an extremely poor prognosis and few effective treatment options. Although ~10% of ACC cases are associated with several cancer predisposition syndromes (such as Li Fraumeni syndrome, multiple endocrine neoplasia type 1, Lynch syndrome, Beckwith–Wiedemann syndrome, and Familial Adenomatous Polyposis), the
majority are thought to be sporadic and their pathogenesis remains poorly understood (1, 2). To date, few risk factors for developing ACC have been identified (3).

Pheochromocytomas and paragangliomas are rare neuroendocrine tumors (estimated prevalence: <0.5% among hypertensives (4)); however, recent genetic advances have revealed that nearly 40% of these tumors arise in patients with pathogenic germline mutations (4, 5). Loss-of-function mutations in the succinate dehydrogenase genes (SDHx) are among the most common causes of hereditary pheochromocytoma-paraganglioma syndromes. The SDH complex on the mitochondrial membrane plays a pivotal role in cellular energy metabolism as it links the Krebs cycle to oxidative phosphorylation (6, 7). It is hypothesized that loss-of-function mutations in one of the SDHx genes (SDHA, SDHB, SDHC, SDHD, SDHAF2) may interrupt the Krebs cycle and result in a state of cellular pseudohypoxia (6) that increases the susceptibility for pheochromocytoma, paraganglioma, renal cell carcinoma, gastrointestinal stromal tumors, pituitary tumors (8, 9, 10), and pancreatic neuroendocrine tumors (11). ACC is not a known manifestation of any SDHx-associated tumor syndrome.

We identified a series of four patients with ACC who harbored somatic mutations in SDHx genes that were also confirmed to be germline mutations in 3 out of 4 of them. The concurrence of pathogenic SDHx mutations in the germline and ACC is expected to be statistically improbable and may herald an important association that suggests ACC may be a rare manifestation of SDHx-related tumor syndromes. Herein we report our patient series to provide insights for future investigations that may elucidate the nature of this association and its implications for patient care.

Subjects and methods

Subjects and cases were identified by physicians caring for ACC patients at two large institutions as part of clinical care. Systemic review of local databases confirmed these cases as the only available cases with germline SDHx variants and a diagnosis of ACC. All patients described herein provided informed consent to participate in the described research and IRB approval was obtained.

Results

Case 1: A 35 year old man presented with abdominal pain and overt physical evidence of the Cushing syndrome,
and was found to have a 15 cm left adrenal mass (Table 1). Family history revealed that his father had a mediastinal paraganglioma at age 40 and a renal cell carcinoma at age 60 (Fig. 1A). The patient underwent radical adrenalectomy with nephrectomy after biochemical evidence excluded a pheochromocytoma. Pathology revealed a high-grade ACC with invasion into the ipsilateral renal parenchyma. Genetic testing was undertaken based on the family history of a paraganglioma and revealed a germline SDHC mutation (c.397C>T, pR133X) in the patient, the patient’s father, and the patient’s asymptomatic daughter. Germline TP53 testing was normal. There was no other family history of ACC or known SDHx-related tumors. Immunohistochemistry demonstrated intact staining for SDHA and SDHB. There was no loss of heterozygosity for SDHC on next generation sequencing of the tumor. Since SDHC inactivation can be induced via non-genomic mechanisms (12, 13), we assessed the function of the succinate dehydrogenase complex using mass spectroscopy on fresh frozen specimens of the ACC and found a normal succinate:fumarate ratio of 4.41 (s.d. 1.45) (14).

The patient was treated with adjuvant mitotane therapy for a high grade stage III ACC. He developed a large recurrence (35 cm) that was treated with surgery. He subsequently received four cycles of etoposide, doxorubicin, and cisplatin (EDP), streptozotocin, and phase I trials for progressive metastatic disease. He died three years following his initial diagnosis. Both his daughter and father are being monitored using a whole-body imaging surveillance program and have not been found to have any new tumors.

Case 2: A 35 year old man presented with hypertension, hypokalemia, and overt signs of the Cushing syndrome. Imaging revealed a large right adrenal tumor with metastases to the liver and lungs. Biopsy of the mass confirmed a low-grade ACC with loco-regional invasion. Genetic tumor testing revealed the c.43C>T, p.R15X mutation in SDHC in the tumor. There was evidence suggestive of likely loss of heterozygosity of the
entire chromosome 1 (as well as other chromosomes), including the SDHC locus with 70% mutated allele reads vs 30% wild-type allele reads by hybrid capture based on comprehensive genomic profiling in the course of clinical care (personal communication, James Sun, Foundation One). The mutation was subsequently confirmed to be present in the patient’s germline. Germline TP53 testing was normal. However, LOH analysis and immunohistochemistry could not be confirmed as no tumor tissue was available. There was no family history of ACC or any SDHx-related tumors (Fig. 1B and Table 1). The patient’s son and father were both found to carry the same SDHC mutation.

The tumor was inoperable due to inferior vena cava invasion. The patient was treated with mitotane and EDP, and subsequently with 5-FU and streptozotocin due to progression of disease. He is currently alive 2 years after initial diagnosis.

Case 3: A 24 year old man presented with Cushing syndrome and was found to have a 15 cm left adrenal mass. Following radical adrenalectomy, pathology revealed a low-grade ACC with vascular invasion and extra-adrenal extension. Genetic testing by a broad multi-gene next generation sequencing panel revealed a germline SDHA mutation (c.91C>T, p.R31X). Germline TP53 testing was normal. Immunohistochemistry showed normal staining for SDHA and SDHB and sequencing of the tumor tissue excluded a deletion as a mechanism of LOH. There was no family history of ACC or any SDHx-related tumors (Fig. 1C and Table 1). He was treated with adjuvant radiation and mitotane therapy and has not had any evidence of recurrence more than two years later.

Case 4: A 40 year old woman presented with abdominal pain and hirsutism and was found to have the Cushing syndrome and severe hyperandrogenism. Imaging revealed a 12 cm left adrenal mass. Following a radical adrenalectomy, pathology revealed a high-grade ACC with local extra-adrenal invasion. Genetic tumor testing using a multi-gene next generation sequencing panel revealed an SDHA mutation (c.1258C>T, p.Q420X) in the tumor without evidence of loss of heterozygosity. Immunohistochemistry showed normal staining for SDHA and SDHB. There was no family history of ACC or any SDHx-related tumors (Fig. 1D and Table 1). She was treated with adjuvant mitotane, and subsequently with EDP, gemcitabine and capecitabine, and nivolumab for metastatic disease. The patient died before germline testing for TP53 and SDHx genes could be performed.

**Discussion**

In summary, we report four patients with ACC who harbored SDHx mutations (corresponding germline mutations confirmed in 3 out of 4), suggesting ACC might be a rare manifestation of SDHx-associated hereditary paraganglioma syndrome.

Although several tumor entities have been described in association with SDHx germline mutations, this is the first case series reporting ACC as a potential manifestation of the hereditary SDHx syndromes. It is recognized that tumors in patients with SDHx mutations may result from loss of heterozygosity leading to disruption of the Krebs cycle, but the downstream mechanisms leading to tumorigenesis are less well understood. Interestingly, only one of our four cases may have developed loss of heterozygosity in the ACC as evidenced by clinical tumor exome sequencing. Importantly, LOH did not occur in a specific fashion, it encompassed the entire chromosome 1 as well as multiple other chromosomes. None of the tumors analyzed showed loss of SDHB or SDHA staining. The fact that this immunohistochemical staining was preserved and biochemical testing in Case 1 did not reveal an altered fumarate to succinate ratio makes a contribution of a disrupted TCA cycle unlikely.

**Table 2** SDHx variants in adrenocortical carcinoma from The Cancer Genome Atlas.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Variant</th>
<th>Mutation type</th>
<th>Exac allele frequency (all)</th>
<th>Exac allele frequency (excluding TCGA)</th>
<th>ClinVar interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDHB</td>
<td>rs34599281</td>
<td>c.A178G:p.T60A</td>
<td>Nonsynonymous</td>
<td>0.00007413</td>
<td>0.00007533</td>
<td>Uncertain significance</td>
</tr>
<tr>
<td>SDHB</td>
<td>rs148738139</td>
<td>c.C247T:p.S85</td>
<td>Synonymous</td>
<td>0.00004132</td>
<td>0.000043</td>
<td>Likely benign/benign</td>
</tr>
<tr>
<td>SDHC</td>
<td>rs182629842</td>
<td>c.*78G&gt;A: 3 prime UTR</td>
<td>3 Prime UTR</td>
<td>0.001667</td>
<td>0.0017</td>
<td>Uncertain significance</td>
</tr>
<tr>
<td>SDHC</td>
<td>rs201210474</td>
<td>c.*84G&gt;C: 3 prime UTR</td>
<td>3 Prime UTR</td>
<td>0.000488</td>
<td>0.00044</td>
<td>Benign/not provided</td>
</tr>
<tr>
<td>SDHA</td>
<td>NA</td>
<td>c.A101G:p.H34R</td>
<td>Nonsynonymous</td>
<td>0.000008236</td>
<td>0.0000014</td>
<td>0</td>
</tr>
<tr>
<td>SDHA</td>
<td>rs200526913</td>
<td>c.G991A:p.A331T</td>
<td>Nonsynonymous</td>
<td>0.00001647</td>
<td>0.00000951</td>
<td>NA</td>
</tr>
<tr>
<td>SDHAF2</td>
<td>NA</td>
<td>c.T8C:p.V3A</td>
<td>Nonsynonymous</td>
<td>0.0007256</td>
<td>0.0068</td>
<td>Pathogenic/benign</td>
</tr>
<tr>
<td>SDHD</td>
<td>rs34677591</td>
<td>c.G34A:p.G125</td>
<td>Nonsynonymous</td>
<td>0.001214</td>
<td>0.0022</td>
<td>Likely benign/benign</td>
</tr>
<tr>
<td>SDHD</td>
<td>rs61734352</td>
<td>c.C312T:p.H104H</td>
<td>Synonymous</td>
<td>0.0007413</td>
<td>0.0007533</td>
<td>Uncertain significance</td>
</tr>
</tbody>
</table>

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Although there remains a chance that the observed variants do not contribute to a potential predisposition to ACC, it is important to mention that all observed variants were premature stop-codons, and are recognized as *bona fide* pathogenic alleles with regards to the established SDHx associated syndromes predisposing to pheochromocytoma and paraganglioma. However, the mutations for Cases 1 and 3 (SDHC c.397C>T and SDHA c.91C>T) have been observed in a larger population database (Table 1), suggesting common low penetrance founder mutations. The SDHA c.91C>T variant occurs in high frequency relative to ACC or any SDHx-related tumors, suggesting a germline prevalence of ~1:6000 and hence very low penetrance. It is important to note that the area surrounding this mutation (±100bp) has a homology of >97% with two SDHA pseudogenes. Although the pseudogene is not reported to carry the variant in question, one cannot exclude the possibility that there is a polymorphism in an SDHA pseudogene rather than SDHA itself. There were no specific reported variants in SDHx in the recent larger series of molecular analysis of ACC (15, 16). However, we reviewed The Cancer Genome Atlas germline data for ACC for variants in SDHx genes (Table 2). There were a total of nine variants of which four are likely benign and the other five of uncertain significance according to ClinVar. No obviously pathogenic variants were found.

We recognize the role of bias in our study since all cases were ascertained in specialized centers with a focus on cancer genetics; therefore, there was likely a higher likelihood that these patients underwent testing using a germline gene panel and/or a research-based next generation testing for somatic variants in the tumor tissue, followed by confirmatory testing for suspicious germline variants. However, considering the rarity of ACC as well as SDHx mutations in the population, the co-occurrence in our cases argues for a true association rather than a chance event. It should be noted, that in addition to our observations, one single case report in 2011 described both ACC and pheochromocytoma in a patient with no known pathogenic germline mutations in SDHB, SDHC, or SDHD (17); however, germline SDHA testing and genetic tumor testing were not conducted. It is important to note that the absolute risk increase for ACC in SDHx mutation gene carriers does not reach a level that would justify targeted ACC surveillance in SDHx mutation carriers; however, ACC should be considered as a potential rare manifestation of SDHx mutations, and the role of SDHx mutations in the pathogenesis of ACC should be further investigated. More systematic somatic and germline testing of patients with ACC in the future may shed further insights into this potential association, and should be required before considering any changes in clinical practice for the surveillance of SDHx pathogenic gene variant carriers or the genetic evaluation of ACC patients. Indeed, while we feel that our observations are extremely interesting to the research community, we would like to point out that we were unable to identify a classical contribution to tumorigenesis of ACC (e.g. absence of LOH, intact immunohistochemistry for SDH components). Therefore, the implications for clinical care and conclusions regarding pathogenesis will require further research to build on our current observations.

In conclusion, we observed a series of patients with ACC that carried pathogenic germline and somatic SDHx mutations. While a simple chance association cannot be excluded, the likelihood of these extremely rare co-occurrences being attributed to simple chance is unlikely. Rather, our observations may represent a serendipitous finding that needs to be further explored to understand novel pathogenic links using detailed tumor analyses, such as methylation patterns and overall chromosomal phenotype of ACCs arising in SDHx pathogenic variant carriers as well as interrogation of a potential pseudohypoxia phenotype and activation of HIF-signalling, which we were unable to perform due to unavailability of sufficient biospecimen.

**Declaration of interest**
The authors have nothing to disclose. There is no conflict of interest regarding the work presented in this manuscript.

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**Author contribution statement**
T E and A V identified the patients and wrote the manuscript. J E, L H, D W, M M and G D H abstracted clinical data. A M L and T L W conducted molecular analysis. All authors reviewed and edited the manuscript.

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