Published in final edited form as:

Cancer Genet Cytogenet. 2009 July 15; 192(2): 82–85. doi:10.1016/j.cancergencyto.2009.04.009.

Loss of heterozygosity of succinate dehydrogenase B mutation by direct sequencing in synchronous paragangliomas

Pinki Prasad^a, Jeffrey A. Kant^d, Marcia Wills^b, Mandy O'Leary^b, Harold Lovvorn III^c, and Elizabeth Yang^{a,*}

^a Department of Pediatrics, Vanderbilt University School of Medicine, 397 PRB, Nashville, TN 37232

^b Department of Pathology, Vanderbilt University School of Medicine, VCH 11223 DOT, Nashville, TN 37232

^c Department of Surgery, Vanderbilt University School of Medicine, VCH 11223 DOT, Nashville, TN 37232

^d Departments of Pathology and Human Genetics, University of Pittsburgh, 3550 Terrace St., Pittsburgh, PA 15261

Abstract

Extraadrenal pheochromocytomas and paragangliomas are rare entities within the pediatric population. We report the presentation of three synchronous extra-adrenal abdominal paragangliomas in an adolescent boy who carries a germline mutation in the succinate dehydrogenase B (*SDHB*) gene. Loss of heterozygosity of this allele was demonstrated by direct sequencing of DNA from two of his tumors, confirming loss of tumor suppressor function in the pathogenesis of these paragangliomas.

1. Introduction

Paragangliomas are tumors that arise from neural crest cells, comprising a group of dominantly inherited disorders characterized by the development of highly vascularized, nonchromaffin tumors arising in parasympathetic ganglia. Paragangliomas can secrete catecholamines, and they are distinguished from pheochromocytomas by their extra-adrenal site of origin. Up to 50% of paragangliomas are familial and occur as paraganglioma syndromes (PGL) [1]. Six susceptibility genes have been identified, including three genes encoding subunits of succinate dehydrogenase (SDH), comprising complex II of the mitochondrial electron transport chain [2–4]. Germline mutation in *SDHD* at 11q23 predisposes to benign head and neck tumors. *SDHC* mutations at 1q21 are associated with solitary tumors. Mutations in the iron-sulfur protein catalytic subunit, *SDHB* at 1p36, commonly lead to extra-adrenal abdominal tumors, classically secreting norepinephrine [2–5]. Approximately 30% of *SDHB* tumors have malignant potential. Paragangliomas are primarily seen in adults, but are increasingly being recognized in children. Pediatric paragangliomas represent early manifestations of hereditary disease due to germline mutations in the von Hippel Lindau susceptibility gene *VHL*, MEN-2-associated *RET* gene, or *SDH* genes. Heterozygous germline SDH mutations resulting in loss of function confer tumor susceptibility. The tumors demonstrate loss of heterozygosity (LOH), thus SDH subunit genes function as classic tumor suppressors.

*Corresponding author: Tel.: 615-936-3585; fax: 615-936-1767. elizabeth.yang@vanderbilt.edu (E. Yang).

Here we report a pediatric patient heterozygous for a *SDHB* mutation who presents with three simultaneous extra-adrenal paragangliomas. Sequencing of DNA from his tumors revealed the absence of the wild-type *SDHB* allele, which is consistent with loss of the tumor suppressor function in the tumorigenesis of this class of paragangliomas.

2. Materials and methods

2.1. Case history

A 13-year-old boy was seen in the surgery clinic for a small incidental nodule on his left chest wall that had already disappeared by the time he came to clinic. Nevertheless, the patient was noted to have a history of profuse sweating, flushing, headaches, lightheadedness, and fatigue exacerbated by exercise. Due to frequent headaches, vomiting, and absence from school, this young man was thought to have behavioral problems, which led to admission into an alternative school. His initial visit to the surgeon revealed hypertension (blood pressure 179/108) and tachycardia (pulse 108), precipitating a computed tomography scan of the abdomen to evaluate renovascular causes of hypertension. The scan showed normal renal vessels but unexpectedly revealed three highly vascular extra-renal, extra-adrenal masses at the level of the renal hilum, as well as a small right renal parenchymal mass and a right renal cyst (Fig. 1). Plasma measurements revealed epinephrine 39 (0–75 pg/ml), norepinephrine 16,519 (75–475 pg/ml), and total plasma catecholamines 16,558 (75–550 pg/mL). Urine measurements showed epinephrine 23 (0–25 $\mu\text{g}/24\text{ hr}$), and norepinephrine: 3,068 (0–90 $\mu\text{g}/24\text{ hr}$). Due to the hypertension, the adrenal masses were thought to be pheochromocytomas. The patient was admitted to the hospital for alpha blockade, lasting 1 month, followed by surgical resection of all three tumors. Five days after surgery, the patient's blood pressure was 115/58, with urine catecholamines nearly normalized at epinephrine 38 $\mu\text{g}/24\text{ hr}$ and norepinephrine 136 $\mu\text{g}/24\text{ hr}$. All symptoms abated after surgery. Four months later, the child's catecholamine levels remained within the normal range and total body MRI detected no tumors.

3. Results

3.1. Tumor histology

The three tumors measured $7.1 \times 6.8 \times 2.8\text{ cm}$, $8.0 \times 3.5 \times 2.5\text{ cm}$, and $4.5 \times 3.5 \times 3.0\text{ cm}$. On hematoxylin and eosin-stained slides, the three lesions were similarly composed of small cohesive nests of rounded cells with speckled nuclear chromatin, infrequent nucleoli, and scattered nuclear polymorphism with occasional larger atypical cells identified. The overall architecture showed a characteristic zellballen appearance with fibrovascular septae surrounding nests of cells, and an intervening sustentacular cell network made of long slender cells with tapered cytoplasmic processes (Fig. 2). These features, in addition to the extra-adrenal locations of the tumors, are consistent with retroperitoneal paragangliomas. Two of the tumors were submitted for DNA sequencing. The small renal parenchymal mass was a nephrogenic adenoma.

3.2. Gene sequencing

The presence of three synchronous tumors suggested a genetic predisposition. DNA from peripheral blood leukocytes was sequenced initially for mutations in the *VHL* tumor suppressor gene and the *RET* oncogene, both of which were normal. Subsequent sequencing of *SDHD*, *SDHC*, and *SDHB* genes revealed a previously reported heterozygous *SDHB* mutation, deletion of a cytosine at nucleotide 88 in exon 2 codon 30, causing a frameshift resulting in a translation stop at codon 76 [6]. (Fig. 3, top, arrow). Sequencing of the *SDHB* gene from the patient's asymptomatic father revealed the identical heterozygous mutation (Fig. 3, bottom).

Exon 2 of the *SDHB* gene was sequenced from paraffin slides of two of the three tumors. Tumor tissues inevitably contain stromal elements and blood supply of normal tissue origin. To minimize contamination by nontumor tissue, DNA was extracted from microscopically identified homogeneous regions of the tumor that were free of visible normal stromal tissue. Sequencing of both tumors revealed the predominance of the nucleotides representing the mutated allele over the diminished peaks of the normal sequence, consistent with loss of heterozygosity of *SDHB* in both tumors (Fig 3, middle).

4. Discussion

Loss-of-function mutations as a cause of familial paraganglioma were first identified in the *SDHD* gene, and subsequently in the *SDHC* and *SDHB* genes [2–4,7]. Mutations in *SDHB*, sometimes referred to as paraganglioma syndrome type 4 (PGL4), are notable for a higher incidence of abdominal tumors and potential for metastasis. Single-nucleotide missense, nonsense, and frameshift mutations, as well as large deletions, have been identified in the *SDHB* gene [2,8,9]. Here we report the identification of a heterozygous c.88delC frameshift mutation in the germline of a child bearing three intra-abdominal, extra-adrenal paragangliomas, as well as in his asymptomatic father, along with corresponding loss of the normal allele (LOH) in the child's tumors by DNA sequence analysis.

This mutation was reported previously in a 12-year-old boy with an abdominal tumor and lung metastasis whose mother also carried the mutation but was tumor-free [6]. Diagnosis of paraganglioma syndrome in a child as the index case, while the mutation-positive parent had not presented with disease, is known and demonstrates variable penetrance of this pathogenic mutation. We measured catecholamines in the father of our patient and found no elevation of plasma epinephrine or norepinephrine to suggest occult tumors. The relatively early presentation of multiple tumors in this child suggests that additional genetic modifiers existing in the child, but not his father, may facilitate increased genomic instability and accelerated loss of the wild-type allele.

Loss-of-function mutations associated with cancer predisposition predict complete loss of tumor suppressor gene function in tumors resulting from loss of heterozygosity at the mutation site. LOH in *SDHB* tumors has been demonstrated by fluorescence in situ hybridization by using probes for chromosome 1 p in a nonfamilial malignant pheochromocytoma [10]. Loss of the wild-type *SDHB* allele in tumor DNA was reported by direct sequencing of a renal cell carcinoma in two individuals [11]. Our demonstration by sequence analysis of the predominance of the mutated *SDHB* allele over the normal allele in both abdominal tumors of this patient confirms loss of LOH as an important feature of paraganglioma pathogenesis in this tumor predisposition syndrome. While renal cell carcinomas are found in paraganglioma syndromes, the renal parenchymal lesion in our patient proved to be a benign adenoma, and is an incidental finding that is unrelated to the paraganglioma syndrome.

The diagnosis of three simultaneous tumors raises the question of whether all three tumors were the result of a single LOH event representing a primary tumor and two metastases, or three separate LOH events occurring close to each other in time. Previous medical records could not be obtained because our patient had switched parental custody and recently moved to the area. From the child's account and sketchy parental history, our patient had been hospitalized in a different city 2 years before for workup of severe headaches and vomiting, including extensive scans that were all unremarkable. Presumably, at least one catecholamine-secreting tumor was present at that time but was below the limits of detection.

References

1. Neumann HP, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M, Buchta M, Franke G, Klisch J, Bley TA, Hoegerle S, Boedeker CC, Opocher G, Schipper J, Januszewicz A, Eng C. European-American Paraganglioma Study Group, Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. *JAMA* 2004;292:943–51. [PubMed: 15328326]
2. Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, Sköldbberg F, Husebye ES, Eng C, Maher ER. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am J Hum Genet* 2001;69:49–54. [PubMed: 11404820]
3. Baysal BE. Clinical and molecular progress in hereditary paraganglioma. *J Med Genet* 2008;45:689–94. [PubMed: 18978332]
4. Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN, Richard CW 3rd, Cornelisse CJ, Devilee P, Devlin B. Mutations in SDHS, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 2000;287:848–51. [PubMed: 10657297]
5. Mora J, Cascón A, Robledo M, Catala A. Pediatric paraganglioma: an early manifestation of an adult disease secondary to germline mutations. *Pediatr Blood Cancer* 2006;47:785–9. [PubMed: 16304664]
6. Benn DE, Croxson MS, Tucker K, Bambach CP, Richardson AL, Delbridge L, Pullan PT, Hammond J, Marsh DJ, Robinson BG. Novel succinate dehydrogenase subunit B (SDHB) mutations in familial pheochromocytomas and paragangliomas, but an absence of somatic SDHB mutations in sporadic pheochromocytomas. *Oncogene* 2003;22:1358–64. [PubMed: 12618761]
7. Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nat Genet* 2000;26:268–70. [PubMed: 11062460]
8. Astuti D, Hart-Holden N, Latif F, Laloo F, Black GC, Lim C, Moran A, Grossman AB, Hodgson SV, Freemont A, Ramsden R, Eng C, Evans DG, Maher ER, et al. Genetic analysis of mitochondrial complex II subunits SDHD, SDHB and SDHC in paraganglioma and pheochromocytoma susceptibility. *Clin Endocrinol (Oxf)* 2003;59:728–33. [PubMed: 14974914]
9. Bayley JP, Devilee P, Taschner PE. The SDH mutation database: an online resource for succinate dehydrogenase sequence variants involved in pheochromocytoma, paraganglioma and mitochondrial complex II deficiency. *BMC Med Genet* 2005;6:39. [PubMed: 16288654]
10. Gimenez-Roqueplo AP, Favier J, Rustin P, Rieubland C, Kerlan V, Plouin PF, Rötig A, Jeunemaitre X. Functional consequences of a SDHB gene mutation in an apparently sporadic pheochromocytoma. *J Clin Endocrinol Metab* 2002;87:4771–4. [PubMed: 12364472]
11. Vanharanta S, Buchta M, McWhinney SR, Virta SK, Peczkowska M, Morrison CD, Lehtonen R, Januszewicz A, Järvinen H, Juhola M, Mecklin JP, Pukkala E, Herva R, Kiuru M, Nupponen NN, Aaltonen LA, Neumann HP, Eng C. Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am J Hum Genet* 2004;74:153–9. [PubMed: 14685938]

Figure 1.

(A) CT scan shows three synchronous and distinct paragangliomas, one on the right (c) and two on left (b and d, on either side of the renal artery). Lowercase letters overlying each tumor correspond with the operative photographs in B–D. (B–D) Operative photographs of each paraganglioma. Note hypervascularity. Ao, aorta; IVC, inferior vena cava, RH, renal hilum.

Figure 2.

(A) Hematoxylin and eosin (H&E; $\times 200$) showing delicate intervening fibrovascular septa surrounding classic nesting, zellballen formation, and sustentacular cells with long, tapered cytoplasmic processes. The nuclear chromatin is diffusely speckled with infrequent nucleoli and cytoplasmic focal vacuoles. (B) H&E ($\times 400$) showing nuclear pleomorphism.

Figure 3.

Dideoxy sequence tracings of SDHB exon 2 in the sense (forward) direction are presented from DNA isolated from the proband's peripheral blood (top), abdominal tumor (middle), and peripheral blood of a first-degree relative (bottom). The normal DNA sequence of exon 2 in this region is indicated in blue between the upper and middle panel with a vertical bar just before the start of the c.88delC frameshift variant. Note that the two peripheral blood tracings are comparable, while the tumor shows relative loss of signal compared to peripheral blood for one nucleotide at each position where the frameshift results in heterozygosity. A blind linear compilation of nucleotides with diminished signal

corresponds to normal sequence in exon 2, consistent with significant loss of this allele in tumor tissue.