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Mutation of SDHB is a Cause of Hypoxia-Related High-Altitude Paraganglioma

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Abstract

**Purpose:** Paragangliomas of the head and neck are neuroendocrine tumors and are associated with germ line mutations of the tricarboxylic acid cycle-related genes SDHB, SDHC, SDHD, and SDHAF2. Hypoxia is important in most solid tumors, and was directly implicated in tumorigenesis over 40 years ago when it was shown that dwelling at high altitudes increases the incidence of carotid body hyperplasia and paragangliomas. Although recent research has now elucidated several pathways of hypoxia in paragangliomas, nothing is currently known of the genetics or of gene-environment interactions in high-altitude paraganglioma. We postulated that SDH mutations might play a role in these tumors.

**Experimental Design:** Patients from a Mexican family, originating and resident in Guadalajara, were tested for mutations of SDHD, and subsequently, for mutations of SDHB followed by immunohistochemical confirmation of SDHB loss.

**Results:** Two patients, born and resident at altitudes of between 1,560 and 2,240 m, were found to have head and neck paragangliomas, including a remarkably aggressive recurrent tumor. Mutation analysis identified a pathogenic missense mutation in exon 7 of SDHB, c.689G>A, p.Arg230His, and loss of the SDHB protein was confirmed by immunohistochemistry.

**Conclusions:** This is the first report of a SDH gene mutation in paraganglioma at high altitude. A rapidly recurrent head and neck paraganglioma is a very rare finding in an SDH mutation carrier, suggesting a gene-environment interaction. Neither patient showed evidence of sympathetic paraganglioma.

Paragangliomas of the head and neck (HN-PGL; also referred to as chemodectomas) are rare, mostly benign tumors of the parasympathetic nervous system. They occur most commonly in the carotid body, the main peripheral sensor of physiologic hypoxia (low oxygen levels). Paragangliomas of the sympathetic nervous system (SPGL) arise in the adrenal medulla or in the extra-adrenal paraganglia of the thorax and abdomen, and are often referred to as pheochromocytomas or extra-adrenal paragangliomas.

A genetic basis for HN-PGL was established with the identification by Baysal et al. (1) of germ line mutations in the succinate dehydrogenase, subunit D gene (SDHD). Other subunits of SDH and related proteins were subsequently shown to be involved in HN-PGL and SPGL (2–4). These genes encode subunits or associated proteins of the succinate dehydrogenase complex, which plays a central role in energy metabolism as both an enzyme of tricarboxylic acid cycle and as complex II of the mitochondrial respiratory chain, involved in oxidative phosphorylation.

In recent years, there has been a revival of interest in metabolic adaptations of solid tumors, specifically the very high rate of glycolysis and decline in tricarboxylic acid cycle activity, often referred to as the “Warburg effect” (5, 6). The Warburg effect is fundamental to a broad range of tumors and is now the basis for the widely used technique of 18F-fluorodeoxyglucose positron emission tomography tumor imaging (7). The discovery of mitochondrial tumor suppressor genes related to deficiencies of the tricarboxylic acid cycle and the respiratory chain, including those encoding succinate dehydrogenase (SDH) and fumarate hydratase, has further stimulated this interest. Mutation of these genes results in tumors, including paragangliomas and pheochromocytomas, cutaneous and uterine leiomyomas, and renal cell cancer (8, 9).
The mutation of subunits of SDH has been shown to result in the accumulation of succinate and the inhibition of hypoxia-inducible factor 1 (HIF-1) prolyl hydroxylases (PHD), leading to the stabilization of HIF-1 (10, 11), a key factor in the hypoxia response (12). Oxygen normally facilitates the degradation of HIF-1 due to hydroxylation by oxygen-dependent PHD and targeting of a VHL-E3 ubiquitin-protein ligase degradation complex. As oxygen levels decline, the HIF-1 protein is no longer degraded and could enter the nucleus to stimulate gene transcription. PHDs are α-ketoglutarate–dependent dioxygenases, and require molecular oxygen and α-ketoglutarate to hydroxylate their targets, generating succinate as a byproduct. The activation of HIF-1 by inhibition of PHDs has been termed “pseudo-hypoxia,” and the effects of succinate on this process could be reversed in vitro by the addition of α-ketoglutarate (13, 14).

In addition, SDH dysfunction also leads to the generation of reactive oxygen species via the inhibition of SDH in its role as complex II of the respiratory chain (15–17). Reactive oxygen species themselves lead directly to the stabilization of HIF-1 (18). These recent mechanistic insights suggest that low oxygen levels have a central role in the genesis of these tumors, and clearly suggest a link to high-altitude paraganglioma.

It was first shown over 40 years ago (19) that humans and various other mammalian species dwelling at high altitudes develop pronounced carotid body hyperplasia and benign tumors (chemodectomas; refs. 20–23). The observation that the main oxygen-sensing organ of the body could give rise to tumors under conditions of low oxygen was the first indication that oxygen could act as an environmental modifier of tumorigenesis. This finding was placed in a molecular context with the identification of mutations in the SDH genes, involved in oxidative phosphorylation, and the recent developments described above have provided further insights into the possible mechanisms driving tumorigenesis.

High-altitude paraganglioma has a prevalence of up to 1 in 10 in humans and almost 1 in 2 in bovines (22, 24), remarkable in light of the low-altitude prevalence of HN-PGL of 1 in 500,000 or less. Although an elevation of 2,000 m has been referred to as marking the border of an increased incidence of nonchromaffin paragangliomas, this is simply due to a lack of inclusion of individuals living at intermediate elevations in published studies (19, 22, 25). The effect of elevation might be detectable at much lower altitudes (26).

The role of the SDH genes in high-altitude paraganglioma cases has received little attention to date and no mutations have been identified (27). SDHB mutations in particular show a striking reduced penetrance (28–30), and might contribute more significantly to this disease than has previously been realized.

The etiologic relationship between hereditary syndromes and high-altitude paragangliomas remains completely unexplored, and the clear environmental component to these tumors has also received little attention. Here, we present suggestive evidence that a gene-environment interaction is strongly modifying the tumor phenotype. Due to recent advances in the field (9), such a gene-environment interaction now has a highly plausible molecular basis, and the study of the molecular mechanisms underlying high-altitude paragangliomas might provide insights relevant to the study of solid tumors in general.

Here, we describe a family with two cases of HN-PGL, including a male with bilateral tumors, originating and resident in Mexico at elevations of between 1,560 and 2,200 m. These are the first cases to link high-altitude paraganglioma to mutations of the SDH genes.

Materials and Methods

Patients

The family was referred to the Hospital de Especialidades CMNO, IMSS, Guadalajara, Jalisco, Mexico, by the family physician. All individuals tested provided informed consent. Tumors were classified following the system of Shamblin et al. (31). DNA extraction from whole blood was carried out using the procedure by Miller et al. (32). The SDHD and SDHB genes were amplified by PCR using standard procedures, and primer sequences are available on request (purchased from Sigma-Genosys). Sequencing was carried out on an ABI 3730 DNA sequencer (Applied Biosystems), and was analyzed using Mutation Surveyor (SoftGenetics). Mutations are described in accordance with the recommendations of the Human Genome Variation Society.
**Results**

**Family**

In a Mexican family originating and still resident in Guadalajara, Jalisco State, two of four offspring in generation II developed carotid body paraganglioma (Fig. 1). The city of Guadalajara lies 1,566 m above sea level, and patient I has also been a long-time resident of Mexico City, situated at 2,240 m above sea level. The parental generation had no symptoms which would indicate the presence of paraganglioma. All nonaffected living descendants currently have no suspicious symptoms.

**Patients**

Patient I, a female, currently 68 years of age (Fig. 1, II-1), originated and is currently resident in Guadalajara, where she lived until the age of 28. She then moved to Mexico City, living there for 25 years, before returning to Guadalajara.

The patient presented at age 67 with bilateral carotid body tumors, with a previous history of ovarian cancer (53 years of age). Presentation was preceded by a 1-year period of clear symptoms including dysphonia, dysphagia, and predominantly right-sided preauricular pain. Angiography of the supra-aortic trunks revealed a classic image (Fig. 2C) resulting in a diagnosis of a left-sided carotid body paraganglioma. The patient was operated to remove the tumor (Shamblin III), which showed mild nuclear atypia and infiltrated the capsule. A second patient, currently 65 years of age (Fig. 1, II-2) and the brother of patient I, also originating and still resident in Guadalajara, had a history of systemic arterial hypertension over the previous 10 years. He presented with a unilateral left-sided cervical mass located in the carotid body that he first noticed at the age of 59, and which developed over a 5-year period. Angiography of the supra-aortic trunks revealed an aggressive growth pattern and within 2 months had reached to the extent seen in Fig. 2B. Despite five sessions of radiotherapy, the volume of the tumor increased, and due to continuous weight loss and compression of neighboring structures, tracheostomy and gastrostomy were done.

Patient II developed carotid body paraganglioma (Fig. 1). The city of Guadalajara lies 1,566 m above sea level, and the brother of patient I, also originating and still resident in Guadalajara, had a history of systemic arterial hypertension over the previous 10 years. He presented with a unilateral left-sided cervical mass located in the carotid body that he first noticed at the age of 59, and which developed over a 5-year period. Angiography of the supra-aortic trunks revealed a classic image (Fig. 2C) resulting in a diagnosis of a left-sided carotid body paraganglioma. The patient was operated to remove the tumor (Shamblin III), which showed mild nuclear atypia and infiltrated the capsule. A second female in the family, 61 years old, is currently unaffected (Fig. 1, II-3). She had an unremarkable medical history including only glaucoma, and hysterectomy due to uterine fibroids. Recent investigations revealed no evidence of a tumor in the head and neck, abdominal, and/or thoracic regions.

The final sister is deceased, at the age of 39, due to lung cancer (Fig. 1, II-4). She had five children and six grandchildren, who are all currently healthy and have not been tested.

The three living members of generation II have undergone abdominal and thoracic computer tomography scanning to detect occult extra-adrenal PGLs and chromocytomas. None were found.

**Mutation analysis**

Because this family presented with HN-PGL, they were initially suspect for mutations of the SDHD gene. No mutations were found in any of the individuals. The SDHB gene was subsequently analyzed. A pathogenic mutation was identified in the SDHB gene in all three living individuals of generation II. A missense mutation in exon 7, c.689G>A, leads to the protein change p.Arg230His (Fig. 3A). Arg230 is a highly conserved amino acid, showing 100% identity between Homo sapiens and various bacterial species including Escherichia coli, Bacillus subtilis, and Vibrio cholerae (Fig. 3B). SIFT (34), PolyPhen (35), and Pmut precalculated (36) analyses all indicate that this is a pathogenic variant. This conclusion is also supported by previous reports of this mutation as a cause of both HN-PGL and malignant sPGL, and that this same residue is also mutated to Cys, Gly, and Leu in other patients with HN-PGL (37).

**Immunohistochemistry**

A formalin-fixed, paraffin-embedded tumor block was available from patient 2 and was used for H&E staining to assess the morphology of the tumor. This revealed histology distinctive for paragangliomas, with clustering of chief cells in typical cell nests surrounded by sustentacular cells and a rich microvasculature (Fig. 4A).
It has recently been shown that SDHB immunohistochemistry on routine formalin-fixed, paraffin-embedded paragangliomas and pheochromocytomas could reveal the presence of SDHB, SDHC, and SDHD germ line mutations with a high degree of reliability (33). We wished to confirm this finding regarding the specific mutation here and to examine whether a high-altitude paraganglioma also showed absence of the SDHB protein. As seen in Fig. 4B and C, staining of SDHB is virtually absent in the SDHB tumor, whereas it was very strong in a non-SDH PGL (Fig. 4C). This is a typical pattern for a tumor carrying a mutation of the SDH genes (33).

Discussion

Familial HN-PGL is a rare condition with a genetic cause related to gene mutations of SDHB, SDHC, and SDHD. Identification of mutation carriers allows increased...
surveillance, and the early detection and treatment of tumors. This potentially allows a reduction in the incidence of surgical morbidity, which is related to the size and extent of the tumor, which may be particularly significant in patients living at high altitude.

Patient 1 in our study experienced an aggressive recurrent tumor, with a striking growth rate and a volume almost equivalent to the original tumor within 2 months of excision. The only published study to address the growth rate of HN-PGL showed an indolent growth pattern with a mean doubling time of 4.2 years (38). Although little is currently known of the recurrence patterns of these tumors, Elshaikh et al. noted a median time to recurrence of 36 months (range, 13-350) but without specifying the extent of the recurrence (39). Interestingly, a recent study described a case of a child with paraganglioma who had previously resided for a number of years at an elevated altitude and experienced a period of rapid tumor growth during a return visit at high altitude (∼1,500 m; ref. 40). Both this case and our patient 1 suggest that tumors under conditions of low oxygen might display an altered growth pattern. The genetic predisposition of our patients is the main explanation for their paragangliomas, but the growth pattern of the recurrent tumor in patient 1 is suggestive of a modifier role for altitude in this tumor. The study of additional high-altitude tumors will allow the possible role of altitude in growth behavior to be evaluated. The close relationship of the mechanisms of hypoxia and the “pseudo-hypoxic” phenotype of SDH-related cases suggest that strong gene-environment interactions could occur in SDH cases at high altitude. Although this is indeed suggested by patient 1 of this study, further careful investigations are needed to confirm this observation.

The relationship of several important facets of paraganglioma tumorigenesis to solid tumors in general has been highlighted by recent studies. Much current interest centers on the role of hypoxia in PGL, the so-called “pseudo-hypoxia,” resulting from the inhibition of SDH and the activation of HIF-1. HIF-1 is a transcription factor complex and key factor in the cellular response to hypoxia (41), and when stable, leads to increased transcription of a battery of genes that mediate an adaptive response to reduced oxygen. The inappropriate activation of HIF-1 might play a role in SDH paraganglioma. The activation of HIF-1 under acute environmental hypoxia proceeds rapidly but little is currently known about the effects of sustained, chronic hypoxia on the activity of HIF-1 in the carotid body (42); therefore, its possible role in the etiology of high-altitude tumors remains to be explored. Equally, other proposed mechanisms of PGL tumorigenesis might also be related to high altitude, including inhibition of developmental apoptosis (43), and adaptations in the metabolism of tumors known as the Warburg effect (44, 45).

A striking aspect of the epidemiology of high-altitude paragangliomas is the sex-dependent risk for development of these tumors, with an 86% to 96% female predominance (22, 25, 46), contrasting with the ∼50:50 ratio seen in low-altitude studies (47). Various explanations have been advanced for this female predominance, including reduced pulmonary capacity in females, periodic blood loss due to menstrual bleeding (25), or a genetic predisposition in Latin populations (46). Alternatively, it might be related to generally lower hematocrit levels in females (36-46% in adult females and 41-54% in adult males), working mechanistically through a relative iron deficiency, as iron has been shown to directly regulate SDHD (Sdh4) mRNA in yeast (48), and to be an essential cofactor for PHDs, again suggesting a possible mechanistic link with SDH defects.

The apparent altitude-dependent bias in gender ratios (22, 25, 46) might be attributable to the greater role of heredity in low-altitude series, as the female/male ratio in nonfamilial, mutation-negative HN-PGL is rather similar at ∼4:1 (47, 49).

Relatively fewer familial cases are seen in Latin series, which might argue for a predominance of cases due to environmental hypoxia. Equally, evidence has accumulated in recent years suggesting that SDHB mutations show reduced penetrance, leading to a sporadic-like presentation...
(28–30); hence, the contribution of SDHB mutations to high-altitude HN-PGL/sPGL could be significant.

Our understanding of the etiology of paraganglioma has shown major advances in recent years and several compelling hypotheses are currently under active investigation. Clearly, both genetic and environmental factors play a role in these tumors. The molecular basis of hypoxia-driven high-altitude paragangliomas remains unknown but recent advances suggest several interesting avenues of investigation. We anticipate that our identification of a genetic basis for some cases of high-altitude paraganglioma might help revive the long-neglected study of high-altitude paragangliomas, leading to both valuable clinical insights into PGL syndromes and further knowledge of common mechanisms of relevance to a wide range of tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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