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Review

Pheochromocytoma in von Hippel–Lindau disease and neurofibromatosis type 1

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Abstract

Clinical and genetic understanding of chromaffin tumors has been greatly enhanced in the last few years. Although some pheochromocytoma genes may still be unknown, the role of *RET*, *VHL*, *SDHB*, *SDHD* and *NF1* genes is unequivocal and phenotypes are also being better characterized. The loss of function of *VHL* and *NF1* genes can lead to a variety of tumors including phechromocytoma and their mechanism of action is under intensive investigation. Many different mutations are responsible for VHL gene inactivation but only missense mutations have been described so far in families with pheochromocytoma. Because of its large size extensive mutation analysis of the *NF1* gene has seldom been performed, and mutations have only been identified in about 15% of patients. Several point mutations have been found in exon 31. Differences in pheochromocytoma phenotype in VHL or NF1 are not very pronounced, but it may be of some interest to consider the two groups separately. In VHL, pheochromocytoma has an earlier onset than in sporadic forms, it is often multiple, and malignancy is less frequent. The mean age of diagnosis is 28 years, the youngest patient being 5 years old. In NF1 patients pheochromocytoma phenotype is similar to sporadic forms. The mean age of pheochromocytoma onset is 42 years; 84% of patients have solitary adrenal tumors, 9.6% have bilateral adrenal disease and 6.1% have ectopic pheochromocytomas; malignant pheochromocytomas were identified in 11.5% of the cases. The group of pheochromocytoma susceptibility genes includes, along with the tumor suppressor genes *VHL* and *NF1*, the proto-oncogene *RET* and the genes encoding succinate dehydrogenase subunit D and succinate dehydrogenase subunit B. Whether there is a common pathway among these different genes is still a matter of debate.

Introduction

Clinical and genetic understanding of pheochromocytoma and paraganglioma has been greatly enhanced in the last few years [1]. The discovery of the role of succinate dehydrogenase gene (*SDHB*, *SDHD*, *SDHC*) mutations has enabled the definition of the pheochromocytoma/paraganglioma syndrome [2]. The analysis of apparently sporadic pheochromocytomas has shown that 10–24% of such patients are carriers of a germ-line mutation of one of the genes responsible for the inherited forms [3]. Although some pheochromocytoma genes may still be unknown [4], the role of RET, VHL, SDHB, SDHD and NF1 genes is unequivocal [5] and phenotypes are also being better characterized [6].

VHL and NF1 are definitely two important anti-oncogenes, their loss of function can lead to a variety of tumors and their mechanism of action is under intensive investigation, since it is a fundamental model for the development of cancer. Differences in pheochromocytoma phenotype in VHL or NF1 are not very pronounced, but it may be of some interest to consider the two groups separately (Table 1).

Pheochromocytoma and von Hippel–Lindau disease

General

Von Hippel–Lindau (VHL) disease is an autosomal dominant familial cancer with an incidence of 1 in 36,000 live births, a penetrance of 97% by age 60 years and a variable inter- and intra-familial expression (OMIM 193300).

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Table 1. Comparison of major characteristics between VHL-associated and NF1-associated pheochromocytoma.

	VHL	NF1
Disease prevalence	1:36,000–1:85,000	1:3,000
Pheochromocytoma prevalence (%)	20–25	0.1–5.3*
Age at diagnosis (years)	30	42
Symptomatic pheocromocytoma (%)	20–30	60–80
Bilateral location (%)	40	10
Extra-adrenal	2–11	6

*3.3–13 at autopsy.

The first link between pheochromocytoma and VHL disease was established in the fifties and it is now evident that pheochromocytoma is part of the disease, with a prevalence of about 20%.

VHL disease predisposes an individual to the development of different types of tumor in bilateral and multicentric forms. Besides pheochromocytoma, retinal angiomas, hemangioblastomas of the central nervous system (mainly cerebellum and spinal cord), renal cysts and clear cell carcinomas, pancreatic cysts, neuroendocrine pancreatic tumors, tumors of the endolymphatic sac and epididymal cystadenoma may develop in various combinations in VHL patients.

Among the main clinical features of VHL disease, the absence or presence of pheochromocytoma has been identified as the element enabling the disease to be classified as type 1 or type 2, respectively. This broad definition has been split further to distinguish between type 2A, without, and type 2B with renal cell carcinoma, the most serious and life-threatening complication of the disease [7–11]. This classification, based on the exclusive occurrence of renal cell cancer on the one hand and pheochromocytoma on the other, needs modification. VHL type 1 is predominantly associated with renal cancer, but not with pheochromocytoma, and vice versa for VHL type 2. Also, families who have so far only shown pheochromocytoma should initially be assumed to have a separate type of VHL, type 2C [12].

The phenotype

Pheochromocytoma in VHL differs from sporadic tumor. In VHL, pheochromocytoma has an earlier onset (19 years earlier), it is often multiple, and malignancy is less frequent. The mean age of diagnosis is 28 years, the youngest patient being 5 years old.

In VHL, as in sporadic form, pheochromocytoma and paraganglioma [13, 14] may cause hypertension or paroxysmal hypertensive crises and symptoms such as headache, palpitation, chest pain, flushing, sweating, postural dizziness, and paleness. However, the frequency of hypertension and symptoms is lower in VHL than in sporadic pheochromocytoma.

Although pheochromocytoma is often asymptomatic in patients with VHL and biochemical tests may also be normal, its 'behavior' is unpredictable: biologically inactive lesions may suddenly become dangerous, or benign pheochromocytomas may become malignant. About 5% of patients with VHL may die of endogenous catecholamine excess from the tumor.

The clinical and biochemical phenotypes of pheochromocytoma in VHL and multiple endocrine neoplasia type 2 patients have been compared and found to have some differences [15]. Generally speaking, pheochromocytomas proved less symptomatic in VHL than in MEN, the frequency of hypertension and other symptoms being 20–30% in VHL and 40–60% in MEN 2 patients.

Phenylethanolamine N-methyl transferase (PNMT) and tyrosine hydroxylase (TH) were differentially expressed in VHL and MEN 2: PNMT and TH expression were found higher in the latter type of pheochromocytoma. While TH is the rate-limiting enzyme in catecholamine synthesis, PNMT converts norepinephrine into epinephrine. This corresponds to a different secretory activity with a higher level of metanephrines (requiring PNMT) in MEN 2 and higher levels of normetanephrine in VHL [15]. The best biochemical test for diagnosing pheochromocytoma would consequently be detection of normetanephrine in VHL and metanephrine in MEN 2 [15].

The genotype

The *VHL* gene is located on chromosome sub-band 3p25–26 and consists of three exons with an open reading frame of 639 nucleotides. The two alternative transcripts, characterized by the presence or absence of exon 2, are ubiquitously expressed in adult tissues but seem to be tissue-specific and developmentally-regulated during human embryogenesis.

The VHL protein (pVHL) has been shown to influence several processes, including cell-cycle control, mRNA stability and the regulation of hypoxia-inducible gene expression [16].

One of pVHL's best characterized functions is the ubiquitylation and proteasomal degradation of hypoxia-inducible factor 1 alpha (HIF-1 alpha). Loss of pVHL function results in loss of HIF-1alpha ubiquitylation and consequent up-regulation of hypoxia-inducible genes. It has also been shown that, in the presence of a particular mutation such as p.L188V (the type 2C VHL mutation), a defective promotion of fibronectin

matrix assembly may contribute to pheochromocytoma pathogenesis in VHL disease [17].

Being a tumor suppressor gene, as in the Two Hit model developed by Knudson for retinoblastoma, the *VHL* gene must have both pairs inactivated before the syndrome becomes manifest. The first inactivating mutation is inherited, but the second is a somatic event. This second event often involves partial or complete wild allele deletion. Most VHL-associated pheochromocytomas show loss of the wild-type allele, suggesting a strong argument for the importance of pVHL loss of function in the pathogenesis of pheochromocytoma [18]. The mechanism of wild-type allele impairment was not recognized in some cases, however, raising the possibility of a different mechanism for the altered function of pVHL in pheochromocytoma as would be a dominant negative effect: in this case, the mutant protein can negatively influence the activity of the wild-type protein encoded by the non-mutated allele [19].

Many different mutations are responsible for *VHL* gene inactivation [9]: large deletions, frame-shift mutations and insertions that result in the formation of truncated proteins, nonsense and missense point mutations. The clinical classification of this disorder seems to identify some correlation with the type of mutation. In fact, deletion/insertion and missense mutations have been found in VHL type 1 families (without pheochromocytoma), while only missense mutations have been described so far in families with pheochromocytoma (VHL type 2). Only one possible mutational hot spot has been identified, at the level of nucleotide 712/713 of the cDNA sequence.

In type 1 VHL, mutations are associated with complete loss of pVHL function. Most of the mutations in patients with type 2 VHL disease have been found to affect the beta domain of pVHL, thus interfering with the fibronectin pathway, leaving the alpha domain intact, which is more crucial to pVHL function, and HIF degradation in particular [19]. This may be the reason for the incomplete loss of function of VHL mutations in Type 2 VHL. Gain-of-function mutations in type 2 VHL has also been suggested, but not demonstrated [14].

Pheochromocytoma and neurofibromatosis type 1

General

Neurofibromatosis type 1 (NF1) is the most common familial disease predisposing to peripheral nervous system tumors, with a prevalence of 1/3,000. The diagnosis of NF1 is currently based on clinical criteria.

Among the tumors that could be related to a loss of *NF1* gene function, pheochromocytoma represents a particularly interesting model. Very strong evidence comes from the observation of an increased rate of tumorigenesis in transgenic mice heterozygous for a mutation of this gene. In particular, pheochromocytomas (which are very rare in wild mice) occur relatively frequently in the NF1-mutated animals and represent the strongest link between experimental and human neurofibromatosis [20].

In addition to cutaneous, nodular or plexiform neurofibromas, other tumors occur more commonly in NF1, including intestinal tumors (with a predilection for the duodenum and ampulla of Vater), malignant gliomas and juvenile chronic myeloid leukemia.

For patients with NF1, the risk of developing benign and malignant tumors, mainly of neuroectodermal origin, is approximately four times as high as in the general population.

The association of pheochromocytoma with NF1 was recognized a long time ago. The estimated prevalence of pheochromocytoma in NF1 is between 0.1% and 5.7%, but this tumor has been found at autopsy in 3.3–13.0% of patients with NF1 [21].

The phenotype

A recent review considered a total of 87 women and 61 men with pheochromocytoma and NF1: the mean age of pheochromocytoma onset was 42 years; 84% of patients had solitary adrenal tumors, 9.6% had bilateral adrenal disease and 6.1% had ectopic pheochromocytomas; malignant pheochromocytomas were identified in 17 of the 148 patients (11.5%) [22].

Hypertension is frequent and may develop at any age. In most cases, the hypertension is essential, but a characteristic NF1 vasculopathy can produce renal artery stenosis, coarctation of the aorta, or other vascular lesions associated with hypertension. A renovascular cause is often found in children with NF1 and hypertension [23, 24]

The genotype

The *NF1* gene is located at 17q11.2 and contains 60 exons. The well-documented function of the *NF1* gene as a tumor suppressor has been attributed to the NF1 GAP-related domain. This region lends the *NF1* gene product, neurofibromin, the function of a Ras–GTPase activating protein, a negative Ras regulator that accelerates the conversion of Ras–GTP to Ras–GDP.

Mutational analysis of *NF1* is still difficult due to the large size of the gene, the lack of mutational hot spots, and the high rate of new mutations. Because of the gene's large size (11 kb of coding sequence extending over 300 kb of genomic DNA, including 60 exons), extensive mutation analysis has seldom been performed, and mutations have only been identified in about 15% of patients. Several point mutations have been found in exon 31. Both loss of heterozygosity and loss of neurofibromin have been observed in pheochromocytomas from patients with and without NF1 [25, 26].

Final considerations

The group of pheochromocytoma susceptibility genes includes, along with the tumor suppressor genes *VHL*

and *NF1*, the proto-oncogene *RET* and the genes encoding succinate dehydrogenase subunit D and succinate dehydrogenase subunit B [3]. Whether there is a common pathway among these different genes is sill a matter of debate. At the present moment, the hypoxia pathway is the best candidate. In fact, at least three, if not all, of the above-mentioned genes may interfere, at various levels, with cell response to hypoxia, and with HIF-1 alpha in particular, which is a key protein in the mechanism of cell response to hypoxia.

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