Molecular and Cellular Biology of Pheochromocytomas and Extra-adrenal Paragangliomas

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The 2004 WHO classification of endocrine tumors [1] defines pheochromocytoma as a tumor arising from chromaffin cells in the adrenal medulla. Closely related tumors in extra-adrenal sympathetic and parasympathetic paraganglia are classified as extra-adrenal paragangliomas. A pheochromocytoma is an intra-adrenal sympathetic paraganglioma. Although arbitrary, this nomenclature serves to emphasize important distinctive properties of intra-adrenal tumors that must be taken into account in clinical practice and research. Those include a lower rate of malignancy (~5% overall, vs ~20% for extra-adrenal paragangliomas associated with the sympathetic nervous system [2]), an often adrenergic phenotype (extra-adrenal paragangliomas are almost always noradrenergic [3]), and a proclivity to occur in association with particular genetic disorders (particularly MEN2) [4]. It can be reasonably argued that this classification may detract from efforts to understand the pathobiology of tumors that, overall, are more similar than different. Pheochromocytomas and extra-adrenal paragangliomas often have similar or identical morphology, share a mostly identical neuroendocrine phenotype, and sometimes have the same genetic predisposition. Nonetheless, the need for consistency dictates that the WHO nomenclature, while imperfect, should be adhered to. At the same time, the biological basis for differences in genotype, phenotype and malignancy based on anatomic site must be resolved.

Efforts to understand the pathobiology of pheochromocytoma/paraganglioma have been spurred by recent advances in genetics and gene expression profiling. New understanding of familial syndromes, especially von Hippel-Lindau disease (VHL) and recently defined familial paraganglioma (PGL) syndromes, has provided a platform from which to explore both familial and sporadic tumors. The current challenge is to relate catalogs of genetic and phenotypic markers to tumor cell biology.

Genetics

Physicians have traditionally been taught to remember the clinical properties of sympathetic paragangliomas according to the "10 per cent rule"- 10% familial, 10% malignant, 10% extra-adrenal. That rule is no longer tenable. Both hospital- and population-based studies have demonstrated occult germline mutations characteristic of familial pheochromocytoma/paraganglioma syndromes in more than 20% of patients presenting with apparently sporadic tumors [5,6], bringing the percentage of tumors with a known genetic basis close to 30%. In addition, the anatomic locations of tumors and their risk of malignancy vary according to the underlying genetic defect.

Hereditary syndromes long known to be associated with development of pheochromocytomas/paragangliomas are Multiple Endocrine Neoplasia (MEN) 2A and 2B, von Hippel-Lindau disease (VHL) and neurofibromatosis type 1 (NF1) due
respectively to mutations of the \textit{RET} proto-oncogene and the \textit{VHL} and \textit{NF1} tumor suppressor genes. The list is now expanded to include familial paraganglioma (PGL) syndromes caused by mutations of succinate dehydrogenase genes \textit{SDHD} (PGL1), \textit{SDHC} (PGL3), and \textit{SDHB} (PGL4), which also appear to function as tumor suppressor genes \cite{7}(syndrome numbers were assigned in order of discovery) (Table 1). In addition, VHL is divided into types 1 and 2, defined by the absence or presence of susceptibility to pheochromocytomas/paragangliomas (TABLE 2). Rates of malignancy range from <3\% for tumors with \textit{RET} mutations to >50\% for those with mutated \textit{SDHB} \cite{6}.

\begin{table}[h]
\centering
\caption{Familial Paraganglioma Syndromes}
\begin{tabular}{|l|l|c|c|c|}
\hline
\textbf{SYNDROME} (chromosome) & \textbf{GENE} & \textbf{ADRENAL} & \textbf{OTHER SYMPATHETIC} & \textbf{PARASYMPATHETIC} \\
\hline
PGL1 & $SDHD$ (11q23) & ++ & & ++ \ \\
\hline
PGL3 & $SDHC$ (1q21-23) & & & ++ \ \\
\hline
PGL4 & $SDHB$ (1p36) & ++ & & ++ \ \\
\hline
\end{tabular}
\end{table}

\*Adapted from WHO bluebook 2004 \cite{1}. In contrast to PGL1 or 4, PGL3 tumors are relatively rare, seldom malignant and seldom multifocal \cite{8}.

\begin{table}[h]
\centering
\caption{Subtypes of VHL Disease}
\begin{tabular}{|l|c|c|}
\hline
\textbf{SUBTYPE} & \textbf{MAJOR TUMOR DISTRIBUTION} \textsuperscript{\circ} & \textbf{PHEOCHROMOCYTOMA OR OTHER SYMPATHETIC PARAGANGLIOMA} & \textbf{RENAL CELL CARCINOMA} & \textbf{HEMANGIOBLASTOMA OR RETINAL ANGIOMA} \\
\hline
1 & & \checkmark & \checkmark \ \\
2A & \checkmark & & \checkmark \ \\
2B & \checkmark & \checkmark & \ \\
2C & \checkmark & & \ \\
\hline
\end{tabular}
\end{table}

\textsuperscript{\circ}Adapted from WHO bluebook 2004 \cite{1}. Parasympathetic paragangliomas occur rarely in VHL disease. Note that pheochromocytomas in VHL 2C occur without any other VHL stigmata.

Somatic mutations of the genes responsible for hereditary pheochromocytomas/paragangliomas are uncommon in tumors that are truly sporadic. The latter are reported to harbor somatic mutations of \textit{RET} in up to~ 10\% of cases and \textit{VHL} mutations in ~4\%. Somatic mutations of \textit{SDHB} or \textit{SDHD} have been reported occasionally in sporadic tumors \cite{9,10} that may be solitary or multiple \cite{9}. A novel
aspect of PGL1 is a mode of transmission that appears to involve genomic imprinting, i.e., tumors occur only after paternal transmission of the mutated gene [7].

Several additional unknown genes apparently confer susceptibility to hereditary pheochromocytomas/paragangliomas in some kindreds. One of two recently identified novel gene loci is on chromosome 2q and appears to be the site of a tumor suppressor gene. Another, on chromosome 16p1, is at or near the locus for hereditary neuroblastoma [11]. Synergy between 2q and 16p is a model suggested to explain inherited tumor susceptibility in some patients. In contrast to other pheochromocytoma/paraganglioma syndromes, tumor susceptibility involving these loci is apparently transmitted as a recessive trait [11].

Sympathetic paragangliomas occur anywhere in the distribution of the sympathetic nervous system from the superior cervical ganglion to the walls of pelvic organs. The distribution of parasympathetic paragangliomas is limited to cranial, cervical and thoracic branches of the vagus and glossopharyngeal nerves. Tumors in patients with MEN2 or neurofibromatosis often arise in or near the adrenal. In contrast, VHL tumors, while predominantly intra-adrenal, are not uncommon in extra-adrenal locations and tumors associated with SDH mutations are predominantly extra-adrenal [6]. SDHD or SDHC mutations are usually confined to parasympathetic paragangliomas in the head and neck, while the combination of sympathetic and parasympathetic paragangliomas is particularly suggestive of a mutation in SDHB [9]. The respective estimated risks of developing pheochromocytoma or paraganglioma range from ~ 1% for patients with neurofibromatosis to ~ 50% for those with MEN2, and risks of the tumors becoming malignant range from <3% for patients with MEN2 to 80% for those with mutated SDHB [6].

While the above mutations predispose to the development of paragangliomas, secondary genetic alterations are probably necessary for tumors to develop. Chromosomal losses involving 1p, 3p, 3q and 11q are frequent in both sporadic and familial pheochromocytomas or paragangliomas. The rate at which these secondary changes accrue may account for the variable risk of developing pheochromocytomas or paragangliomas in hereditary tumor syndromes and the nature of the changes may contribute to differences in tumor phenotype. The most prevalent abnormality identified to date is a deletion of a portion of chromosome 1p, also common in neuroblastomas, containing an unidentified tumor suppressor gene [2]. Although the putative suppressor gene locus is close to SDHB, the latter does not appear to be the target [12]. Similarly, the VHL locus is close to the deleted region of 3p. Specific patterns of secondary genetic changes segregate with specific syndromic mutations, suggesting multiple pathways of tumorigenesis as recently reviewed by Dannenberg et al [13]. For example, 1p deletions occur in approximately 80% of MEN2 or sporadic tumors [14]. However, they only are found in 15% of VHL tumors, which more frequently show losses on chromosome 11 [15], as also reported in sporadic parasympathetic paragangliomas [16].

Gene Expression Profiling
Microarray-based gene expression profiling studies complemented by immunohistochemical and/or biochemical analyses have revealed sets of markers that tend to be clustered in tumors with specific genetic backgrounds, in subsets of sporadic tumors, and in benign versus malignant tumors. Two studies now show that pheochromocytomas/paragangliomas with VHL, SDHB or SDHD mutations form a cluster with a “transcription signature” characterized by genes associated with hypoxia-driven transcription pathways. In contrast, the signature of tumors with with RET or NF1 mutations includes features consistent with increased activity of the Ras-mediated MAPK pathway [6,17]. An additional distinctive characteristic of VHL tumors is that they usually do not express phenylethanolamine N-methyltransferase, the enzyme that synthesizes epinephrine from norepinephrine, and are therefore noradrenergic. In contrast, MEN2 and NF1 tumors produce both epinephrine and norepinephrine [18]. As with other genotype-phenotype correlations, care must be taken in these analyses to account for characteristics of the anatomic site of origin of the tumor in question, for example, that epinephrine production is normally confined to the adrenal medulla. In the case of VHL, even intra-adrenal tumors have a noradrenergic phenotype. They are also reported to often have a distinct histological appearance suggestive of hypoxic signaling, with a thick vascular capsule and many small blood vessels interspersed among tumor cells [19].

Cell Biology:

The meanings of differences

Hypothetically, several models might be invoked to explain the genotype-phenotype correlations that characterize pheochromocytomas/paragangliomas:

Cell of origin

Adrenal chromaffin cells of most species have separate populations of epinephrine (E) and norepinephrine (NE) cells that can be readily identified by immunohistochemical staining for PNMT. An attractively simple model would suggest for example that pheochromocytomas in VHL disease or MEN2 arise from such populations. Most (though not all) chromaffin cells in the human adrenal appear to have a mixed phenotype, making that explanation unlikely. Nonetheless, more subtle expressions of cellular heterogeneity may be involved. In the adult human adrenal subsets of medullary cells differentially express various neuropeptides [20] or the RET proto-oncogene [21]. Recent developmental studies of mouse models suggest that sympathoadrenal progenitors are to some extent heterogeneous prior to entering the adrenal primordium and that cell fate commitment within the developing adrenal might involve several different signaling mechanisms [22-25].

Pathway dependence

A model based entirely on pathway dependence would suggest that signal transduction pathways activated by a tumorigenic mutation, e.g., a RET mutation in MEN 2, determine both the distribution of tumors and characteristics of those tumors. Although pathway dependence undoubtedly is involved in genotype-phenotype differences,
determining the precise roles of different pathways either in tumorigenesis or phenotype determination is difficult.

Considerable evidence demonstrates that major signal transduction pathways such as the MAPK cascade can function as “rheostats” rather than “on/off switches” [26]. Signaling effectors can exert qualitatively as well as quantitatively different effects depending on levels of pathway activation. Those levels are determined by the amount of incoming signal (e.g., the concentration of a growth factor [27]), the amount of effector expressed in a target cell (e.g., growth factor receptor), the intracellular distribution of the effector (e.g., cytoplasm or membrane lipid rafts [28] and the expression of cooperative or counteracting signal transducers. Different experimental conditions or models can therefore lead to different conclusions. In cultures of normal rat chromaffin cells, nerve growth factor (NGF) can stimulate both proliferation and terminal neuronal differentiation, both requiring MAPK activation, but the former occurring at 1/10th the NGF concentration of the latter [29]. Expression of constitutively active MEN2 mutant RET is mitogenic in fibroblast cultures but is anti-mitogenic and induces neuronal differentiation in a seemingly more appropriate model of rat pheochromocytoma cells [30]. Physiological activation of wild type ret is by its ligand, glial cell line derived neurotrophic factor (GDNF), also induces neuronal differentiation of mouse [31] and human [32] pheochromocytoma cells.

Functional and anatomic context

Although adrenal chromaffin cells and the chief cells of other sympathetic and parasympathetic paraganglia are closely related and there is some functional overlap [33], their utilization in different anatomic contexts subjects them to different types of stimuli. For example, the adult adrenal medulla responds principally to signals derived from pre-ganglionic sympathetic neurons via trans-synaptic stimulation, while extra-adrenal sympathetic paraganglia are sparsely innervated and presumably respond to local or humoral messengers. The chief cells of the carotid body form reciprocal synapses with glossopharyngeal nerve endings and function in chemosensory reflexes including oxygen-sensing. Even if cells in all three locations were intrinsically identical, their exposure to potential tumor promoter effects of neurotransmitters or other secretagogues would be different, so that a potentially oncogenic mutation expressed in all cells would be manifest by preferential development of tumors in different locations.

In fact, all three of the above probably contribute to genotype-phenotype correlations, although their precise contributions are almost entirely unknown. Intrinsic cell- or tissue-specific differences in expression of critical signal transducers could confer susceptibility to anatomically dependent signals. Nerve endings containing different neurotransmitters [34] could confer functional heterogeneity within cell populations that appear homogeneous.

A further overlay that must be considered is phenotype plasticity. Recent studies of the rat adrenal medulla suggest that neurally derived signals may increase the expression of receptors that regulate chromaffin cell function, including the receptor tyrosine kinase RET. The finding that RET expression is not static may help to resolve the conundrum of how that molecule, which is expressed at very low levels in the adult adrenal, contributes
to the development of adrenal medullary hyperplasia and pheochromocytoma in adults with MEN2 syndromes [35].

The search for similarities: Cross-talk, common denominators and unifying hypotheses

Signaling pathways, receptors and ion channels involved in the function and development of the adrenal medulla and related tissues cross communicate at multiple levels. For example RET encodes a developmentally regulated receptor tyrosine kinase involved in formation and maintenance of the nervous system. Gain-of-function RET mutations in MEN2A cause constitutive kinase activation, one consequence of which is activation of Ras signaling. NF1 encodes a tumor suppressor gene, the functions of which include Ras GTPase activity that may terminate Ras signaling. Loss-of-function NF1 mutations in neurofibromatosis might therefore increase Ras signaling as in MEN2A. VHL encodes a protein that guides proteolytic degradation of hypoxia-inducible transcription factors (HIFs) and other proteins marked for destruction by oxygen-sensitive prolyl hydroxylases, and loss-of-function VHL mutations mimic hypoxia by interfering with this oxygen-sensitive proteolysis. SDH enzymes are components of mitochondrial complex 2 and function both in the Krebs cycle, converting succinate to fumarate, and as components of the electron transport chain. Loss-of-function mutations of SDH hypothetically could mimic hypoxic signaling both through accumulation of succinate and from buildup of reactive oxygen species. However, succinate accumulation may be more important in that oxygen-sensitive prolyl hydroxylases that mark proteins for VHL-guided degradation utilize alpha-ketoglutarate (2-oxoglutarate), the Krebs cycle precursor of succinate, as a co-substrate, generating succinate as a by-product. Excess succinate may cause competitive inhibition of those hydroxylases, thereby indirectly affecting the functions of VHL [36,37]. Hypoxia can also activate p42/p44 MAPK, which are downstream effectors of Ras signaling, through several pathways [33,38]. These effects could occur both in sympatheitic and parasympathetic paragangliomas, as shown by the fact that experimental pheochromocytoma cells express the same oxygen-sensing mechanisms as carotid body chief cells [33].

The concept of cross talk provides a general framework for understanding how mutations of apparently unrelated genes might lead to the same type of tumor by convergent signaling. In addition, they suggest that targeted therapies might be directed either at a specific mutated gene or at a downstream signal transducer. However, it remains to be determined what precise mechanisms lead to tumor formation. In an intriguing recent paper, Lee et al propose that the mutations of RET, NF1, VHL and SDH in hereditary pheochromocytoma/paraganglioma predispose to tumor formation by causing defective developmental culling, promoting survival of damaged cells that would normally destroyed by apoptosis during embryogenesis [39]. An important argument in favor of survival rather than mitogenesis as the major common denominator of the syndrome-associated genes is the rarity of the same mutations in sporadic pheochromocytomas/paragangliomas, suggesting that the mutations only need to act during a limited developmental window. The proposed model was based on studies of
PC12 rat pheochromocytoma cells and the specific convergence point was a prolyl hydroxylase known as EglN3, thus far not found mutated or overexpressed in human pheochromocytomas/paragangliomas. Nevertheless, other genes might serve the same purpose. An important implication of the model is that tumor precursor cells could theoretically be identified and eradicated in the adrenal glands or paraganglia of individuals who carry RET, NF1, VHL or SDH mutations.

Interestingly, the survival promoting effect of mutant VHL in the model proposed by Lee et al does not appear to be predominantly HIF-dependent [39]. This model is thereby the first to adequately account for the fact that VHL2C mutations do not prevent HIF degradation, serving as a reminder that tumor suppressor genes should not forever be conceptually pigeonholed according to the functions intitially ascribed to them as was the case for VHL and HIF. A similar caveat might be applied to NF1 in that it is one of the largest genes in the entire genome and has mutations distributed throughout its length rather than in discrete hotspots as for RET, VHL or SDH. Most NF1 mutations are distant from the Ras-GTPase domain, and evidence of abnormal Ras signaling has not been detected in pheochromocytoma cell lines from nf1 knockout mice [40].

References