The late 19th and early 20th centuries witnessed a remarkable series of discoveries relating to the function and structure of these endocrine glands. They were recognized as being compound endocrine organs consisting of the mesodermally derived cortex and the neural crest derived medulla. Epinephrine (adrenaline) was discovered and shown to be the major catecholamine of the medulla while glucocorticoids, mineralocorticoids and sex steroids were shown to be of cortical origin. During this same period, a wide array of hyperfunctional and hypofunctional states were discovered and their pathological correlates were identified. As suggested by Dr. Sommers, the adrenals really proved to be glands of adventure.

While many of the mysteries of the glands have been resolved, many questions remain. For example,

- Are traditional morphological and immunohistochemical parameters useful in the distinction of benign and malignant adrenocortical neoplasms?
- How useful are comparative genomic hybridization and gene expression profiling in the categorization of benign and malignant adrenocortical neoplasms?
- Can we reliably predict the malignant potential of pheochromocytomas and paragangliomas?
- What are the roles of the multiple endocrine neoplasia (MEN) 2, von Hippel Lindau (VHL) and succinate dehydrogenase genes in the development of pheochromocytomas and paragangliomas of both heritable and sporadic types?
- Do other genetic alterations play a role in the development of pheochromocytomas and paragangliomas?

This symposium will attempt to address some of these questions.

“...the adrenal glands are glands of mystery and adventure” (Sheldon C. Sommers, 1971)

The adrenal glands have had a long and complex history, and for many years, different schools of anatomy doubted their very existence. Although some authors suggested that the first description of the glands occurred in the Bible (Leviticus 3:4), most authorities credit Bartholomew Fracastorius (1520-1574) with the discovery of the adrenal glands in humans. In his great work, Ornithologiae, Fracastorius provided the following description of the adrenals which he referred to as glandulae renibus incandescentes:

“...even if many will consider sufficient what we have said about the surface of the kidneys, somebody could object that I have neglected something and I consider it indicated to say something of the glands, diligently overlooked by other anatomists. Both kidneys are capped on the externally towards the cavity by a gland...one, if he is not very attentive, does really overlook them, as if they were not present...early anatomists and those who write ample treatises on this art in our days failed to detect them. They, pretending to the exact, stand so obstinately for their own and their masters’ errors that often they seem to be fighters rather than searchers of anatomical truth.”

Eustachius’ work had many detractors. Archangelo Piccolomini, the man who advanced the concept that the testicles collect sperm from all parts of the body, wrote “Sometimes one may see two or more glands lying on the kidneys but we do not think that they deserve special attention because they are not found in every case and have no flesh or parenchyma of their own...they might be considered renal excrescences. Why do they exist in a few? The same way superabundance of material creates a sixth finger, they originate from a seminal surplus and that is the way they come out of the kidneys.”

Casper Bartholin (1611) referred to the glands as capsulae atrabiliares and wrote that “they are to be found in all bodies, whatever. Archangelus (who compares them to the sixth finger) may say...we call them, because of the function we attribute to them, the capsules of black bile...they contain a cavity, which holds a dark and facelulent liquid. Their use is still unknown.” Johann Vesling upheld this view when he wrote, “What their use is, the human mind has not conjectured.”

It took nearly 200 more years after Bartholin’s description to prove that the glands were solid and that their hollow appearance resulted from autolysis. Numerous hypotheses concerning their function appeared during this time including the notions that they served as accessory kidneys, reservoirs of black bile, producers of meconium and as “tissue fillers” to separate the kidneys from the diaphragm. Because of vascular connections with the testes and ovaries, it was also suggested that the adrenals played a key role in sexual function. Vaalsvra is quoted as saying, “I think they produce in male and female a liquid which is necessary for fecundation.” With the later discovery of the sex steroid hormones, Vaalsvra’s hypothesis seems remarkably prescient.

References


Histopathology and Immunohistochemistry of Adrenal Medullary Tumors and Paragangliomas.

Anne Marie McNicol, Reader in Pathology, University of Glasgow, Royal Infirmary, Glasgow, Scotland, UK

Introduction
Paragangliomas are of two types- sympathetic and parasympathetic. The former arise from the sympathetic plexus in the posterior and paravertebral and para-aortic axis in close relation to the sympathetic trunk, and include the adrenal medulla and the organs of Zuckerkandl. Adrenals arise from the neural crest in the connective tissue adjacent to pelvic organs. Parasympathetic paragangliomas are found in the head and neck close to vascular structures and branches of the pharyngeal and vagus nerves and include carotid body and ganglionic, intravagal and jugulotympanic tumors. These tumors have in the past been given a variety of names. However, the recent WHO classification views them as follows. Pheochromocytoma is a tumor of chromaffin cells of the adrenal medulla and all others are ‘extra-adrenal paragangliomas’ (1). Paragangliomas may occasionally be a primary site for other tumors such as ganglieneuroblastoma, neuroblastoma and schwannomas. These components may form a component of the rare composite pheochromocytoma (2) or paraganglioma.

Some aspects of pheochromocytoma and paraganglioma
Paragangliomas are rare, with an estimated incidence of 1:300,000 (3). Sympathetic tumors occur most frequently in the adrenal medulla and organs of Zuckerkandl and often give rise to hypertension related to excess secretion of catecholamines. Jugulotympanic and carotid body paraganglia are almost always benign and present with symptoms related to compression of surrounding structures. Symptomatic paragangliomas (4) and these do not usually result in endocrine syndromes. About 90% of symptomatic paragangliomas occur in adults and 90% of these are intra-adrenal. In children, the tumors are more commonly extra-adrenal and multifocal than in adults (5). About half of the extra-adrenal sympathetic tumors arise in the organs of Zuckerkandl, with most of the remainder in the retroperitoneum. There is an equal sex distribution, except in children and in patients with von Hippel Lindau (VHL) disease, where males are more commonly affected (6). Carotid body paraganglioma is more common in men peaking at high altitude, most likely in response to hypoxic stimuli. It now appears that familial disease is much more common than the previous figure of 10% with the identification of germline mutations in the subunits of the succinyl dehydrogenase enzyme (7) that compose Multiple Endocrine Neoplasia type 2 (MEN2), Neurofibromatosis type 1 and von Hippel Lindau (VHL) syndrome (4). This is beyond the scope of this review.

Pheochromocytoma
Pheochromocytoma usually secretion epinephrine and have well-recognized clinical correlations, often presenting with symptoms of catecholamine hypersecretion. However, a significant number of are autonomic or asyndromic and do not present with symptoms. Similarly, a small number of histological features (21) with malignant tumors having a score of four or more. This requires further validation in an extended series and testing on extra-adrenal tumors.

It should be noted that intertumoral correlation of tumors with carbohydrate in paragangliomas of the adrenal medulla does not predict malignant potential.

A number of studies have addressed the issue of correlation of pheochromocytoma with malignant potential, in general based on the calculation of a Ki-67 index using the antibody MB-1 (22, 23) and some have suggested a positive correlation between high proliferation rates and malignant behavior. However, there is no agreement on the level of cutoff, values between 2% (24) and 10% (21) used. In addition, using thresholds of 2.5% (20) and 5% (23) there was only 95% sensitivity. Some of these discrepancies may relate to different methods used and these will have to be addressed to allow a valid assessment of the utility of this technique.

A number of other markers have been reported to correlate with malignancy (25) including head shock protein 90, N-cadherin, vascular endothelial growth factor and breakdown products of choromagran (26). None of these has yet found its way into diagnostic practice.

References
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 22q 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 kindreds. One of these loci, 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 RET proto-oncogene and the VHL and NF1 tumor suppressor genes. The list is now expanded to include familial paragangliomas (PGL), syndromes caused by mutations of succinate dehydrogenase genes SDHD (PGL1), SDHC (PGL3), and SDHB (PGL4), which also appear to function as tumor suppressor genes [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 pheochromocytoma/paraganglioma (Table 2). Rates of malignancy range from <3% for tumors with RET mutations to >50% for those with mutated SDH (B).

### TABLE 1. FAMILIAL PARAGANGLIOMA SYNDROMES

<table>
<thead>
<tr>
<th>SYNDROME</th>
<th>CHROMOSOME</th>
<th>GENES</th>
<th>TUMOR DISTRIBUTION</th>
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<tr>
<td>ADRENAL</td>
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<td>PARASYMPATHETIC</td>
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<tr>
<td>PGL1</td>
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<td>SDHD (11q23)</td>
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</tr>
<tr>
<td>PGL3</td>
<td>11q22-23</td>
<td>SDHC (11q23)</td>
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</tr>
<tr>
<td>PGL4</td>
<td>11q22-23</td>
<td>SDHB (11q23)</td>
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</table>

*Adapted from WHO bluebook 2004 [7]. Pheochromocytomas occur in VHL disease. Note that pheochromocytoma in VHL, AD, and NF1 are all different.

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 RET in up to 10% of cases and VHL mutations in ~4%. Somatic mutations of SDH or SDHB have been reported occasionally in sporadic tumors (9,10) that may be solitary or multiple [9]. A novel

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 RET or NF1 mutations includes features consistent with increased activity of the Ras-mediated MAPK pathways [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 nonadrenergic. 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 nonadrenergic 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].

### Table of VHL Disease

<table>
<thead>
<tr>
<th>SYMBYTE</th>
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<tbody>
<tr>
<td>PARAGANGLIOMA</td>
<td>MAJOR TUMOR DISTRIBUTION</td>
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<tr>
<td>RENAL</td>
<td>HEMANGIOBLASTOMA</td>
</tr>
<tr>
<td>NEPHRONOMASTOMA</td>
<td>DIFFERENTIAL DIAGNOSIS</td>
</tr>
<tr>
<td>2A</td>
<td>2B</td>
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</tbody>
</table>

| 2A | 2B | 2C |

Adapted from WHO bluebook 2004 [7]. Pheochromocytomas occur in VHL disease. Note that pheochromocytoma in VHL, AD, and NF1 are all different.

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 sympathetic 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 unambiguously 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 “rehearsal” rather than “on/off switches” [26]. Signaling effectors can exert qualitatively as well as quantitatively different effects depending on levels of pathway activation. These 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 raft) and [28] the expression of cooperative or counteracting signal transducers. Different experimental conditions or models can therefore lead to different conclusions. In cultured normal chromaffin cells, growth factor (NGF) can stimulate both proliferation and terminal neuronal differentiation, both requiring MAPK activation, but the former occurring at 1/10 the NGF concentration of the latter [29]. Expression of constitutively active MER2 mutant RET is a mouse, in embryonic cultures but is anti-angiogenic and induces neurite 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), and oxytocin neuronal differentiation of mouse [31] and human [52] 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, their utilization in different anatomic contexts subjects them to different types of stimuli. For example, the adult adrenal medulla responds specifically to signals derived from paraganglionic sympathetic nerve 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 effectors of neurotransmitters or other secretogogues would be different, so that potentially oncogenic mutation expressed in all cells would be manifest by preferential development of tumors in different locations.

In fact, all of the three above probably contribute to genotype-phenotype correlations, although their precise contributions are almost entirely unknown. Intricately 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 homogenous.

A further overlap 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 glial 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 terminal differentiations are 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, in cultured normal chromaffin cells a prolyl hydroxylase known as EglN3, thus far not found in human paraganglioma or pheochromocytoma susceptibility. Clin Endocrinol (Oxf) 59:728-33, 2003 7. Determining the precise roles of different pathways either in tumorigenesis or phenotype determination is difficult. In an intriguing recent paper, Lee et al propose that the mutations of RET, NF1, VHL, and SDH in hereditary pheochromocytoma/paenganglioma predispose to tumor formation by causing defective developmental cutting, promoting survival of damaged cells that would normally be 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 pheochromocytoma/paenganglioma, suggesting that the mutations need to act during a limited developmental window. The proposed model was based on studies of

Familial Pheochromocytoma and Paragangliomas: an overview.

Vesna Peric and Paul Komminoth

Department of Surgery, University of Zürich and Institute of Pathology, University of Basel, Switzerland

Familial Pheochromocytoma and Paragangliomas (PCCs and PGLs) are rare, often inherited diseases with a broad clinical spectrum. PCCs and PGLs are rare, often inherited diseases with a broad clinical spectrum. The disease with the most dominant inheritance is characterized by medullary thyroid carcinoma, C-cell hyperplasia, hyperparathyroidism and PCCs or PGLs occurring in about 50% of MEN2A and MEN2B patients. PGLs are less frequent, and the prevalence of the disease is unknown. The disease occurs in young adults and is characterized by multiple neuroendocrine tumors, which can be detected by imaging studies.

Familial PCC and PGL: Genotype-Phenotype correlations

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
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<tr>
<td>Multiple endocrine neoplasia type 2 (MEN2)</td>
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<tr>
<td>Multiple endocrine neoplasia type 1 (MEN1)</td>
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<td>Von Hippel-Lindau disease (VHL)</td>
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Families Developing PCCs (VHL Type 2) often show misery mutations of the VHL gene opposed to those with PCC without PGL (VHL Type 1). In those cases, the mutation is often missense opposing to the different tumors.

References


PCC/PGL-Genotype (PC1-PC4):

- PC1: SDHA-associated tumors (PC1, OMIM #153100) are often catecholamine secreting extra-adrenal abdominal or rarely thoracic tumors. Adrenal PCCs may also arise, often in combination with extra-adrenal tumors. SDHA-associated tumors are often malignant, having an aggressive course.
- PC2: SDHB-associated tumors (PC2, OMIM #602537) are familial with germline mutations in the SDHB gene. PC2 are predisposed to PGLs of the head and neck region. SDHB-associated tumors often occur at a young age compared to sporadic PGLs. Rarely thoracic or abdominal catecholamine secreting tumors on sporadic PGLs can develop in addition to the head and neck tumors.
- PC3: SDHC-associated tumors (PC3, OMIM #602538) are familial with germline mutations in the SDHC gene. PC3 are predisposed to PGLs of the head and neck region. SDHC-associated tumors are often multiple and occur at a young age compared to sporadic PGLs. Rarely thoracic or abdominal catecholamine secreting tumors on sporadic PGLs can develop in addition to the head and neck tumors.
- PC4: SDHD-associated tumors (PC4, OMIM #602539) are familial with germline mutations in the SDHD gene. PC4 are predisposed to PGLs of the head and neck region. SDHD-associated tumors are often multiple and occur at a young age compared to sporadic PGLs. Rarely thoracic or abdominal catecholamine secreting tumors on sporadic PGLs can develop in addition to the head and neck tumors.

Familial PCC and PGL: Genotype-Phenotype correlations

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Histopathology and Immunohistochemistry of adenocortical adenoma and carcinoma

Hironobu Sasano, M.D., Ph.D.
Department of Pathology, Tohoku University School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Japan

The major diagnostic problems which surgical pathologists face in adenocortical pathology at this juncture are whether resected lesions are benign or not and if malignant, whether cortical or non-cortical. In this brief review, these two areas will be covered including the potential contribution of immunohistochemistry on this differential diagnosis.

I. Discerning malignancy in adenocortical tumors

When the patients with adenocortical mass are detected, the most important clinical aspect in the management of these patients is whether adenoma mass represents malignant or not. The most important and critical point in adenocortical pathology is therefore the differential diagnosis between adenocortical adenoma and carcinoma.

I-1 Macroscopic Evaluations

When evaluating malignancy of adenocortical neoplasms, gross or macroscopic evaluation is very important. Firstly, the weight of the neoplasm should be determined as carefully as possible. In our experience, the tumor with more than 100g in weight comprised 90% of carcinoma but only 6% of the adenoma. However, it is also very important to note that small adenocortical tumor can metastasize and some large tumors do not. Therefore, the weight of the tumor is very important in evaluating malignancy of adenocortical neoplasms but, as expected, the weight itself is not a reliable prognostic indication of the resected adenocortical tumor. The next important thing is the macroscopic features of the cut surface of the tumor. Ominous hemorrhage and necrosis are rarely observed in adenocortical adenoma. Necrosis is sometimes associated with cystic changes. The presence of necrosis and hemorrhage, therefore, strongly indicates the diagnosis of adenocortical carcinoma. However, it is also true that many adenocortical neoplasms were not associated with foci of necrosis and hemorrhage. In addition, it is also important to sample the specimens from the areas adjacent to the foci of necrosis and hemorrhage when grossing the specimens.

I-2 Histopathological Evaluations

An increasing number of small adenocortical neoplasms has been discovered with the development of CT and MRI scan. Therefore adenocortical carcinomas not associated with these ominus macroscopic features above have recently increased in number. The distinction of those "well-differentiated" adenocortical carcinoma from adenoma could be one of the most diagnostic difficulties in surgical pathology practice. There are no single histological criteria which can reliably differentiate adenocortical carcinoma from adenoma like capsular or vascular invasion of thyroid follicular carcinoma. Only the systems which evaluated multiple histological and/or non-histological criteria of the resected cases can provide reliable histological diagnosis. Among various criteria used, the criteria proposed by Weiss, which requires only histopathological findings and evaluated nine histological features of the tumors most frequently associated with poor clinical outcome of the patients, have been most widely employed due to the straightforwardness of the criteria and easy applicability. These nine histological criterias are as follows: 1. High nuclear grade, 2. Mitotic figures more than 5/50 hpf, 3. Atypical mitotic figures, 4. (Eosinophilic or compact tumor cell cytoplasm >75% of tumor cells), 5. Diffuse architecture (>33% of tumor), 6. Necrosis (contiguous necrosis), 7. Venous invasion - smooth muscle in wall, 8. Sinusoidal invasion (no smooth muscle in wall) and 9. Capsular invasion. In the report of Weiss, 20 of 28 cases that fulfilled three histologic criteria died of disease and it is currently considered that the tumors which met more than three of these criteria should be considered adenocortical carcinoma. The system is straightforward and relatively easy to use, and a good correlation was observed between results and clinical outcome of the patients including the tumor of 15g. However, it is also true that the tumors which behaved not in a malignant fashion in their postoperative course, including the cases of adenocortical oncocytoma were considered as adenocortical carcinoma, although these adenocortical oncocytomas may recur or metastasize in a long period of time. In addition, among these nine criteria, we experienced that nuclear grade, architecture and cytoplasm were likely to be subjective, i.e., the interobserver differences were relatively marked unless observers were well-informed prior to histological examination of adenocortical tumor.

I-3. Potential contribution of immunohistochemistry in differentiation between adenocortical adenomas and carcinomas

At this juncture, only the analysis of cell proliferation using Ki67 or MIB1 and topoisomerase IIalpha antibodies can provide any additional meaningful information to carefully performed histopathological analysis. The adenocortical neoplasms with more than 5-6% of MIB1 labeling index can be considered adenocortical malignancy. However, the same precautions such as fixation, intratumoral heterogeneity and interobserver differences should be noted in applying MIB1 LI in differential diagnosis.

II. Differential diagnosis between adenocortical and non-adenocortical origins.

In the patients who do not manifest any clinical hormonal abnormalities, the malignancies that may be associated with histopathologic differential diagnosis of adenocortical carcinomas at both primary and metastatic sites are renal cell carcinoma, hepatocellular carcinoma, clear cell carcinoma of the ovary and uterus, malignant melanoma, and large cell carcinoma of the lung and pleomorphic carcinoma. Diagnosis of adenocortical carcinoma is important in these patients because of the history of treatment results in a small but significant increase in mean survival times in the patients with adenocortical carcinoma. Among these tumors, the two most important primary neoplasms in the differential diagnosis of primary adenocortical carcinoma are renal cell and hepatocellular carcinoma, especially when the lesions are large. Careful histological examination and detection of biological features of these tumors can resolve the diagnostic dilemma between adenocortical carcinoma and other tumors in most cases. However, it is also true that specific adenocortical tumor marker can contribute greatly to the differential diagnosis of these tumors.

A4BP/SF-1 is a transcription factor of all steroidogenesis and exclusively expressed in steroidogenic cells except for stromal cells in spleen and some gonadotrophic cells in anterior pituitary glands A4BP/SF-1 immunoreactivity was demonstrated in almost all the tumor cells of adenocortical carcinoma, both histological sections and cytology specimens regardless of the degrees of differentiation, but not in renal cell carcinoma, hepatocellular carcinoma, malignant melanoma, ovarian and uterine clear cell carcinoma, large cell carcinoma of the lung and pleomorphic carcinoma. An antibody against A4BP/SF-1 which may be of use in surgical pathology is currently commercially available. Application of A4BP/SF-1 immunohistochemistry can greatly contribute to the differential diagnosis of adenocortical carcinoma from other malignancies both at primary and metastasis sites even in the evaluation of needle biopsy specimens.

References


The Li-Fraumeni syndrome has been linked to mutations of the TP53 gene (19). Many studies have examined TP53 mutations in ACTs with quite variable results (19-33). Generally mutations were found in 20 to 67% of ACCs and were rare in ACAs. While the highest mutation frequency reported in ACCs was 67%, this is not high enough to be used clinically to accurately separate benign and malignant tumors. LOH studies hold the greatest diagnostic potential, as shown in the following figure.

Other mutations have been associated with ACTs (reviewed in (34)) such as mutations of the MEN1 (15-37) and PRKAR1A (38-41), but they have not been demonstrated to have any utility in separating ACAs from ACCs.

Molecular Profiling Studies

One of the difficulties or limitations of using genotyping in a clinical setting is that several different types of mutations can inactivate (or activate) the same gene. Point mutations can be distributed across large genes (i.e., point mutations of MEN1) making their identification technically difficult, and it is also possible to mutate a single gene via distinct mutational mechanisms. For example, the most common type of BRF4 mutation in papillary thyroid carcinoma is a point mutation, but less common translocations and amplifications have also been reported (42-44). Thus, a method based on DNA sequencing for point mutations will completely fail to identify the other mutations. For this reason, as well as others, there is much excitement over high-throughput methods to examine gene expression in tissues (45-49). The development of commercially-available DNA microarrays has permitted their use in a variety of clinicopathologic studies and the first such studies of adenal cortical tumors have been published.

In a study from our laboratory (13), we used oligonucleotide arrays and small cohort of normal adrenal cortex. ACAs and ACCs identify a gene expression profile that robustly separates benign and malignant tumors, including one low-grade tumor. Using a large group of variably expressed genes selected without reference to pathologic diagnosis, separation of ACA from the cortical tissues was observed. The one low grade ACC (designated C13) was intermediate in its classification. When a reduced gene list of differentially expressed genes was used, C13 clearly segregated with the other ACCs. This was observed when both Principal component analysis (PCA) and hierarchical clustering was performed and is shown in the figure below.
References


Collectively, these 2 studies demonstrated clear differences in gene expression between benign and malignant adrenal cortical tumors and illustrate the potential power of molecular profiling approaches based on gene expression for tumor classification.

Future Directions

It is difficult to predict the specific path this field will take in the coming years, especially given the rapid evolution of high-throughput technologies to comprehensively examine various aspects of tumor cell biology. However, it is clear that the fields of Oncology and Pathology are poised to undergo a transformation in which more targeted therapies become available and more intelligent therapeutic choices are made, largely driven by molecular profiling-based assessments of patient’s tumors. It will be exciting to witness and participate in this transformation as it is applied to the diagnosis, prognosis and treatment of adrenocortical carcinoma.