Sporadic Pheochromocytoma: Anapathologic, Immunohistochemical and Cytogenetic Aspects Associated with the Occurrence of Metastasis: A Literature Review

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Abstract

The safe distinction between benign and malignant pheochromocytomas is still an unsolved problem from the standpoint of the pathological diagnosis. In most cases, the decision is thus based on the occurrence of metastasis as the only unambiguous evidence of malignancy. This paper reviews the literature and shows that, although there are positive correlations between anatomopathological, immunohistochemical and cytogenetic findings, the independent analysis of each factor does not establish the ultimate prognosis of malignancy. It is therefore suggested an integrated wide analysis of all factors in an attempt to predication of the metastatic potential of sporadic pheochromocytomas, especially when there is a positive correlation between all the criteria, particularly in the detection of 5% or more of positive nuclei for MIB-1 or CD44 -S negative immunostaining, or both. When 10 or more chromosomal changes are noticed in comparative genomic hybridization, malignancy should be strongly suggested.

Introduction

Pheochromocytomas are derived neoplasms of medullary chromaffin cells of the suprarenal region. Even today, they still pose a great challenge to modern pathology, because we cannot confirm the diagnosis of malignancy only based on morphological analysis. The confirmation of malignancy comes from the occurrence of metastasis (MAITRA et al. 2010).

Recently many researchers throughout the world have demonstrated the correlation between molecular analyses and capacity, or not, of producing metastasis, which may represent an important tool in aid of the distinction of sporadic metastatic and non metastatic pheochromocytomas (AUGUST et al. 2004).

The main signs and symptoms displayed by people with pheochromocytomas are due to excessive release of catecholamines. About 90% of the patients have been diagnosed with arterial hypertension, often resistant to conventional treatment, but being able to respond to the use of α-adrenergic receptors, calcium channel blockers and sodium nitroprusside. A classical triad is accepted, consisting of headache, profuse sweating and heart palpitations in the clinical diagnosis of neoplasms (GIFFORD JR; MANGER; BRAVO, 1994).

The aim of this paper is to review the literature about the main data relating to pathological, cytogenetic and immunohistochemical aspects associated with the occurrence of metastasis in sporadic pheochromocytomas.

Literature review

2.1 Embryology of the Adrenal gland

Adrenal glands, also called adrenals, both sit on the top of each kidney. If cutting the gland, it is noticed that it is encapsulated and divided neatly in two concentric layers, a cortical and a medullary. These layers can be considered two distinct bodies, they have different embryological origins. The cortex has as its origin the coelomic epithelium (mesodermic), and the marrow comes from neural crest cells (neuroectodermal), both with distinct morphology and functions (JUNQUEIRA; CARNEIRO, 2008). The colony-forming cells of the cortex, in the fetus, derive from the posterior abdominal wall mesotelial coat. The cells that originate from the spinal cord derive from an adjoining sympathetic ganglion which, in turn, derives from the neural crest. The mesothelium, arise from the cells of the cortex (the differentiation of cortical areas begins at the end of the fetal period). Still in the cortex, the zona glomerulosa and zona fasciculata are already present at birth, while the reticulated zone is only present in the third year (MOORE; PERSAUD, 2008).

2.2 Pheochromocytomas

The pheochromocytoma is a rare neoplasm with chromaffin cell origin, produces and metabolizzante of catecholamines. About 95% of chromaffin cell neoplasms, catecholamines, occur in the medulla of
the adrenal gland (MCCLELLAN; HARRY; LINEHAN, 1999). However, there are chromaffin cells located in regions outside of the medullary region of the adrenal glands, especially in sympathetic ganglia of the autonomic nervous system, which can cause cancer related to the pheochromocytoma either genectically or functionally, also called paragangliomas. There are also paragangliomas with source in non-chromaffin cells of the parasympathetic nervous system ganglia, which may be adjacent to the aortic arch, in the neck or at the base of the skull and that are designated according to their location. The latter, unlike what happens with the pheochromocytomas and chromaffin cells paragangliomas, have only produced catecholamines in about 5 cases (FITZGERALD, 2007).

The persistent hyper secretion of catecholamines by chromaffin cells of the tumor leads to an excess of the capacity of storage in vesicles and their accumulation occurs in the cytoplasm. Catecholamines suffer from the action of the intracellular metabolism, but its excess and its metabolites diffuse to the circulation and are responsible for a set of metabolic and cardiovascular effects. The pheochromocytomas and the paragangliomas secreting catecholamines are responsible for about 0.1 -1% of all cases of secondary hypertension and by 0, 1% all new cases of hypertension that arise annually (MCCLELLAN; HARRY; LINEHAN, 1999, FITZGERALD, 2007).

The diagnosis of pheochromocytoma is obtained by biochemical tests or blood in urine that reveal excess of catecholamines or its metabolites (COSTA; GARCIA, 2008).

2.3 Relationship between Pheochromocytoma and Genetic Syndromes

Most often, the pheochromocytoma is an unusual and sporadic tumor. However, it can present itself as a genetic pathology, with autosomal dominant inheritance of high penetrance, occurring alone or associated with other diseases (HERMANN; Apud MORNEX, 1964. PEREIRA et al. 2004; MAITRA 2010). The genetic causes of pheochromocytoma include multiple endocrine neoplasia type 2 (NEM2), Von Hippel-Lindau disease (VHL), neurofibromatosis type 1 (NF1) and Familial Paraganglioma Syndrome and pheochromocytoma isolated (table 1).

2.4 Chromaffin Cell Tumor Pathology

The pheochromocytomas are neoplasms formed by chromaffin cells, which synthesize and release catecholamines and, in some cases, peptide hormones. It is important to recognize these tumors because they are a rare cause of surgically correctable hypertension. Traditionally, the pheochromocytoma has been associated with a “rule” of 10 (MAITRA, 2010).

2.4.1 “Rule” of 10

1. Ten percent of pheochromocytomas are extra-adrenal, occurring in places such as the organ of Zuckerkandl and the carotid body. The pheochromocytomas that have developed in the extra-adrenal paraganglios are called para-gangliomas.

2. Ten percent of sporadic adrenal pheochromocytomas are bilateral. This configuration can occur in up to 50% of the cases that are associated with family syndromes.

3. Ten percent of adrenal pheochromocytomas are biologically malignant defined by the presence of metastatic disease. It is noted that the malignancy is more common (20% - 40%) in extra-adrenal paragangliomas and in tumors that arise from the condition of certain germline mutation.

4. Ten percent of adrenal pheochromocytomas are not associated with hypertension. From the 90% who have presented hypertension, approximately 2/3 have presented paroxysmal episodes associated with the sudden increase in blood pressure and heart palpitations, which can occasionally be fatal (MAITRA, 2010).

2.4.2. Macroscopic Aspects

Morphologically, the pheochromocytomas vary from small circumscribed lesions confined to adrenal up to large hemorrhagic masses weighing kilograms. The average weight of a pheochromocytoma is 100 grams, but weights above 1 g up to 4000g have already been described. Larger tumors are well demarcated by connective tissue and medullary or cortical tissue. Fibrous trabeculae richly vascularized in the tumor produce a lobular pattern. In many tumors, the remnants of the suprarenal gland can be seen, lying on the surface or adhered to a pole. The cut surfaces of the minor pheochromocytomas are brownish-yellow. The bigger lesions tend to be haemorrhagic-yellow. They usually destroy the adrenal gland. The fresh tissue incubation with a potassium dichromate solution changes the tumor to a dark brown color due to the oxidation of the stored catecholamines (MAITRA, 2010).

Algust et al. (2004), when analyzed 43 pheochromocytomas, associated the weight and the occurrence of metastasis. All tumors, presented in this study, weighed between 3 and 1800g. Tumors that had greater weight, average of 395, presented
metastasis sometime in the course of the disease. This weight was significantly higher when compared to those tumors that have not metastasized, which had an average of 75 g of weight. The authors still make an important association with the metastatic potential, when 75% of metastatic pheochromocytomas weigh higher than 100 g. Therefore, it makes it clear that tumors, which present more than 100 g, offer greater risk of metastatic potential.

2.4.3 Microscopic Aspects
The histological pattern in pheochromocytoma is very variable. The tumors are composed of polygonal and fusiform chromaffin cells or main cells, grouped with sustentaculares cells in small nests or alveoli (“zellbalen”) by a rich vascular network. Rarely the dominant cell type is a small cell or fusiform. Multiple patterns can be found in any tumor. The cytoplasm has a distinctly granular appearance, but well demonstrated with silver staining, due to the presence of granules containing catecholamines. The cores are generally round to ovoid, with a dotted chromatin shaped as "salt and pepper", which characterizes neuroendocrine tumors. Electron microscopy reveals variable numbers of electrondense secretory granules surrounded by a membrane. The immunoreactivity for neuroendocrine markers (chromogranin and synaptophysin) is seen in the main cells, while the peripheral sustentacular cells get color with antibodies against (S-100), a calcium binding protein expressed by a variety of types of mesenchymal cells (MAITRA, 2010).

2.4.4 Limitation of morphological analysis
The determination of malignancy in pheochromocytoma can be difficult. There is no histological feature that reliably predicts clinical behavior. Several histological characteristics such as number of mitoses, tumor necrosis factor confluent and fusiform morphology, were associated with a considered aggressive behavior and an increased risk of metastasis, but they are not fully trusted. Tumors with benign histological features may give metastasis, while very pleomorphic tumors may remain confined to the adrenal gland. In fact, nuclear and cellular pleomorphism, including the presence of giant cells and mitotic figures, are often seen in benign pheochromocytomas, although the cellular monotony is paradoxically associated with an aggressive behavior. Even the capsular and vascular invasion can be found in benign lesions (MAITRA, 2010). Therefore, the definitive diagnosis of malignancy in pheochromocytomas is based exclusively on the presence of metastasis. These may involve regional lymph nodes, as well as more distant ones, including liver, lungs and bones (SALMENKIVI et al., 2001; MAITRA, 2010).

2.5 Malignancy determined by histopathological criteria
Thompson (2002) while systematically analyzing 100 pheochromocytoma cases, of which 50 showed malignant evolution and 50 with benign evolution, proposed a scale called Pheochromocytoma of the Adrenal Gland Scale (PASS) able to determine malignancy only based on conventional histological criteria. This scale takes into account the following pathological criteria and its respective score (Table 2).

In cases where the score is equal to or greater than 4 (PASS >=4), potentially malignant behavior should be considered. Tumors with score less than 4 (PASS<=4) have benign behavior (THOMPSON, 2002). The author also mentions the fact that no study had yet been conducted this way and that subsequent studies using the scale could help elucidate the accuracy of this system.

2.6 Comparative Genomic Hybridization
It is a molecular cytogenetic method with the potential to detect chromosomal disorders previously inaccessible, since only the DNA is required for the procedure. It is possible to study small amounts of DNA prepared from a few cells with the techniques of polymerase chain reaction (PCR). It is used especially in the detection of tumor suppressor genes and proto-oncogenes (VERMA; BABU, 1995).

This procedure is based on the markup with different colors for the DNA Test or tumorous (green) and for the normal DNA used as control (red). The two samples are mixed and hybridized with normal metaphase chromosomes. If the test sample contains more DNA of a particular chromosomal region than the sample-control, this region is identified by an increase in green fluorescence compared to the red one, featuring a gene amplification. Similarly, a deletion in the tested sample is identified as a reduction in green fluorescence compared to the red one (VERMA; BABU, 1995).

In a research paper performed in Germany, researchers, while studying the clinical course of 43 patients, demonstrated that metastatic pheochromocytomas showed significantly more genetic alterations than the cases without any occurrence of metastasis. Therefore, it has been noted that tumors with 10 chromosomal aberrations or more have shown metastasis in 100% of the cases (n=8). Tumors that have presented eight genetic changes (n= 14) demonstrated occurrence of metastasis in 85.7% of the cases (AUGUST et al., 2004). The
authors also noted that losses on chromosome 9p have only occurred in metastatic tumors.

2.7 Immunohistochemistry

The immunohistochemistry was developed with researches on immunopathology which started in the 1940’s. Only after 1974, when it was possible to demonstrate some tissue antigens by immunoperoxidase technique in tissue fixed in formalin and included in paraffin, Immunohistochemistry was accepted as a simple and practical method in surgical pathology diagnostic routine. The development of monoclonal antibodies, which led to a huge source of highly specific reagents for the demonstration of various tissue or cellular antigens, and the advent of antigen recovery were milestones in the evolution of Immunohistochemistry. It was such a breakthrough in contribution and application of Immunohistochemistry in surgical pathology that the phenomenon started to be known as the Brown Revolution of Histopathology Laboratory (LEONG; WRIGHT, 1987; BODEY, 2003, revised by WERNER, 2005).

2.7.1 Cluster differentiation 44 (CD 44)

The CD44 Antigen is a cell surface glycoprotein involved in cell-cell interactions, cell adhesion and migration, working with a receptor for hyaluronic acid and can also interact with other ligands, such as osteopontin, collagens and matrix metalloproteinases (MMPs). In human beings, the CD44 antigen is encoded by the CD44 gene on chromosome 11 (SPRING et al., 1988). CD44 is expressed in a large number of types of mammalian cells. The standard isoform, called CD44-S, is expressed in most cell types, including exons 1-5 and 16-20. The CD44 variants containing the variable exons called CD44-V. Some epithelial cells also express the isoform (CD44-E), which includes the exons 8-10 (GOODISON; URQUIDI; TARIN, 1999).

In a study conducted in Israel in the year 1998, it was demonstrated strong membranous marking of CD44-S in cells of the medulla of normal adrenal glands, evidenced by strong membranous coloring. Furthermore, it was also demonstrated positivity in cases of pheochromocytoma and adrenalcortical adenomas. The authors conclude that cytoplasmic staining of CD44-S facilitates the diagnosis of cortical adenomas, while membranous positive staining characterizes pheochromocytoma. Therefore, pheochromocytoma, which is probably originated in the neural crest, maintains its expression of CD44. In addition, the strong membranous markup for CD44 in pheochromocytoma can help distinguish adrenalcortical adenomas from other tumors (BARSHACK et al. 1998).

August et al. (2004), while analyzing 43 cases of sporadic pheochromocytomas, related the significant increase in marking of CD44-S in pheochromocytomas to benign clinical course, when compared to the tumors that have developed metastases during the period of clinical evolution.

2.7.2 Ki-67 Antigens and MIB-1

The Ki-67 antigen is described as an excellent the proliferative activity marker, but its use is restricted to fresh-frozen material (CATTORETTI, 1992). Its action is strictly associated with cell proliferation. During interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, while in mitosis most of the protein is transferred to the surface of the chromosomes. Ki-67 is a protein presented during all active phases of the cell cycle (G1, S, G2, and mitosis, but it is absent in resting cells (G0) (SCHOLZEN, 2000). The MIB-1 is a monoclonal antibody produced by recombinant Ki-67 with equivalent feature, being resistant to formalin. Its clinical applicability is to determine, through markup, the Ki-67 index (CATTORETTI, 1992).

In a study with 43 sporadic pheochromocytomas, it was verified significant differences in MIB-1 nuclear marking of metastatic and non-metastatic tumors. The authors describe that 5% or more of the markup was associated with malign clinical evolution in 85% of cases. On the other hand, 100% of non-metastatic tumors showed less than 5% of nuclear markup for MIB-1 (AUGUST et al., 2004).

2.7.3 Tenascin

Tenascin is a significant extracellular matrix glycoprotein. Its function is associated with the regulation of adhesion, differentiation, growth and cell migration. These glycoproteins are expressed during the various stages of development, in the process of healing, in non-neoplastic pathological processes and tumorigenesis (SCHNYDER, 1997).

In a study performed in Finland, researchers proposed that tenascin is associated with malignant potential in pheochromocytomas. A significant difference was observed between malignant and benign pheochromocytomas in a study with 37 tumors. All malignant pheochromocytomas expressed strong or moderate stromal tenascin, while most benign pheochromocytomas showed no or only weak immunopositivity (SALMENKIVI et al., 2001). The authors also suggest that, in addition to tenascin being associated with malignant transformation and metastasis of pheochromocytomas, it would also be a
potential marker for predicting the greater aggressive behavior in these tumors.

Discussion

Despite the differentiation between benign and malignant lesions of sporadic pheochromocytomas is still a problem, and being based on the occurrence of metastases, many researches have demonstrated the correlation between various factors that help distinguish between tumors with metastatic capacity or not. Many works demonstrate the association between anatomopathological findings, cytogenetic and immunohistochemical with the occurrence of metastases, in order to generate data that can serve as a substrate in malignant characterization of sporadic pheochromocytomas (SALMENKIVI et al., 2001; THOMPSON, 2002; AUGUST et al., 2004).

It is possible that cytogenetic analysis in conjunction with immunohistochemistry can be of greatest value in assessing metastatic potential of sporadic pheochromocytomas. According to August et al. (2004), all malignant tumors showed 10 or more chromosomal aberrations and were characterized by complete negative reaction with antibodies against CD 44-S. Also, 62.5% of these tumors showed more than 10% nuclear markup for MIB-1. From this observation, it may be suggested that the missing of CD 44-S molecules related to cell adhesiveness (SPRING et al., 1988) may be related to the ability of stromal invasion, capsular and metastasis formation in regional lymph nodes and other organs.

Aigust et al. (2004), while analyzing 43 pheochromocytomas, associated the weight and the occurrence of metastases. Tumors that have presented greater weight showed metastasis sometime in the course of the disease. 75% of metastatic pheochromocytomas weighed more than 100 g, which may implies that tumors that weigh more than 100 g offer higher risks of metastatic potential.

Conclusions

1. Pheochromocytomas are rare tumors of the sympathetic adrenal system, whose malignancy is still not possible to predict with clarity.
2. Combined analysis among anatomopathological, immunohistochemical and cytogenetic findings provides an important aid in the distinction of benign and malignant sporadic pheochromocytomas.
3. More studies are necessary in different areas of knowledge, particularly in cytogenetic area, in order to elucidate the prognosis of sporadic pheochromocytomas.

References

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Illustrations

Illustration 1

Table 1: Hereditary Syndromes associated with Pheochromocytoma Adapted from MAITRA, 2010.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Mutated Gene</th>
<th>Associated Lesion</th>
<th>Other Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN1</td>
<td>RET</td>
<td>Pheochromocytoma</td>
<td>Medullary Thyroid Carcinoma, parathyroid hyperplasia</td>
</tr>
<tr>
<td>MEN2A</td>
<td>RET</td>
<td>Pheochromocytoma</td>
<td>Medullary Thyroid Carcinoma, Marfanoid Habitus</td>
</tr>
<tr>
<td>VHL</td>
<td>VHL</td>
<td>Pheochromocytoma, Paraganglioma (ipsilateral)</td>
<td>Renal cell Carcinoma, Homer syndrome, Pancreatid Endocrine Neoplasm</td>
</tr>
<tr>
<td>RET</td>
<td>NF1</td>
<td>Pheochromocytoma</td>
<td>Neurofibromatoso Optic Glioma</td>
</tr>
<tr>
<td>Paraganglioma Syndrome Familial</td>
<td>SDH/SDHAF1, SDHAF2, SDHB</td>
<td>Pheochromocytoma Paraganglioma</td>
<td>...</td>
</tr>
</tbody>
</table>

Illustration 2

Table 2: Pheochromocytoma of the Adrenal Gland Scale according to Thompson (2002):

<table>
<thead>
<tr>
<th>Microscopic Feature</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular invasion</td>
<td>1</td>
</tr>
<tr>
<td>Capsular invasion</td>
<td>1</td>
</tr>
<tr>
<td>Periadrenal adipose tissue invasion</td>
<td>2</td>
</tr>
<tr>
<td>Cell nests of large proportions or Diffuse growth</td>
<td>2</td>
</tr>
<tr>
<td>Focal necrosis or confluent</td>
<td>2</td>
</tr>
<tr>
<td>High cellularity</td>
<td>2</td>
</tr>
<tr>
<td>Cellular Monotony</td>
<td>2</td>
</tr>
<tr>
<td>Mitotic figures &gt; than 3 in 10 fields of great increase</td>
<td>2</td>
</tr>
<tr>
<td>Atypical mitotic figures</td>
<td>2</td>
</tr>
<tr>
<td>Marked Nuclear Pleomorphism</td>
<td>1</td>
</tr>
<tr>
<td>Hyperchromasia</td>
<td>1</td>
</tr>
</tbody>
</table>
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