Composite Pheochromocytoma

A Clinicopathologic and Molecular Comparison With Ordinary Pheochromocytoma and Neuroblastoma

Jessica M. Comstock, MD, Carlynn Willmore-Payne, MT(ASCP), Joseph A. Holden, MD, PhD, and Cheryl M. Coffin, MD

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Abstract

Composite pheochromocytoma is a rare adrenal tumor composed of ordinary pheochromocytoma and other components, most frequently neuroblastic elements. Little is known about its biologic potential, therefore creating a clinical dilemma on diagnosis. This study investigates the clinical characteristics and N-myc amplification status of 4 cases of composite pheochromocytoma and compares them with selected cases of ordinary pheochromocytoma and neuroblastoma. The age range of the patients with composite pheochromocytoma was 15 to 40 years with an equal M/F ratio, including 2 patients with syndromes. None of these composite pheochromocytomas demonstrated N-myc amplification, none recurred, and there were no deaths. Of the classic pheochromocytomas, none demonstrated N-myc amplification, 2 recurred, and there were no deaths. Of the neuroblastomas, 5 (50%) of 10 showed significant N-myc amplification, and there were 4 known recurrences and 5 known deaths. These findings suggest that composite pheochromocytoma may be regarded as a histologic variant of classic pheochromocytoma.

Ordinary pheochromocytoma (OP) is an uncommon tumor of the adrenal medulla composed of polygonal to spindled cells arranged in an alveolar, trabecular, or solid pattern, often with a typical Zellballen appearance. Approximately 10% are familial, and approximately 10% are malignant. Extra-adrenal pheochromocytomas are termed paragangliomas.

Neuroblastoma is a small round blue cell tumor of childhood. Neuroblastoma is the most immature of the neuroblastic tumors; the others are ganglioneuroblastoma and ganglioglioma. These tumors are differentiated based on the amount of schwannian stroma and the presence or absence of ganglion cell differentiation. The presence of N-myc amplification in neuroblastomas is widely understood to indicate a poor prognosis.

Composite pheochromocytoma (CP) is a rare tumor composed of typical pheochromocytoma and other components, most often neuroblastoma, ganglioneuroblastoma, or ganglioglioma. Rare cases have displayed pheochromocytoma with other coexisting neural or neural crest–derived tumors such as malignant peripheral nerve sheath tumor. Little is known about the biologic potential, outcome, or molecular genetic profile. The pathologic diagnosis of CP creates a clinical dilemma because it is not known whether the neuroblastic component results in therapeutic and prognostic implications different from those in OP.

The purpose of this study was to retrospectively investigate the pathologic features, N-myc amplification status, and clinical characteristics of CP. Given that CP is composed of OP and neuroblastoma, we also compared it with selected cases of OP and neuroblastoma.
Materials and Methods

Case Selection and Review

Four cases of CP were identified from the surgical pathology files at the Primary Children’s Medical Center, Salt Lake City, UT, and the University of Utah Health Sciences Center, Salt Lake City, during a 15-year period (1990-2004). Also, 10 selected samples of each OP (from 9 patients) and neuroblastomas were used for comparison. To highlight the rarity of CP, during the same 15-year period, the 2 institutions had a combined total of 199,319 surgical cases, 599 neuroblastomas, 345 adrenal glands, and 35 OPs.

All selected cases were reviewed by 2 pathologists (J.M.C. and C.M.C.) and classified according to International Neuroblastoma Pathology Classification Criteria9,10 and other standard pathologic criteria.

N-myc Analysis

Tissue sections cut at 10 µm and mounted on glass slides were deparaffinized and lightly stained with H&E. The tissue sections were then subjected to laser capture microdissection (LCM) using the Pixcell IIe Laser Capture Microdissection Instrument and CapSure Macro LCM Caps (Arcturus, Mountain View, CA). Areas of interest were identified using the microscope and a 10× objective. For the OPs and neuroblastomas, these were representative areas of tumor; for the CPs, the areas of interest were the neuroblastic elements. A CapSure Macro LCM Cap coated with a thermoplastic film was placed over the target. An infrared laser was activated by the push of a button, causing the thermoplastic film on the cap to adhere to the target cells. Parameters for LCM on the Pixcell instrument were a laser diameter of 7.5 µm, laser power of 45 to 65 mW, and laser pulse duration of 3.5 to 5.0 seconds. Approximately 200 to 300 laser pulses per specimen were used to capture the regions of interest. The cap containing captured cells was fitted into a 0.5-mL microcentrifuge tube containing 25 µL of Proteinase K digestion buffer. DNA was isolated following procedures previously described.11

Real-time monoplex polymerase chain reaction was performed on a LightCycler, Roche Applied Science, Indianapolis, IN, on the isolated DNA samples, using primers specific for IF-2 and N-myc, as previously described.11 An N-myc/IF-2 ratio greater than 2.2 was considered amplified.

Results

The clinical and pathologic features and N-myc amplification results for CP are summarized in Table 1. The N-myc amplification results for all tumors are listed in Table 2.

Composite Pheochromocytoma

Four cases of CP were identified, all of which contained neuroblastic elements in various stages of differentiation Image 1. The age of patients at diagnosis ranged from 15 to 40 years, and there was equal distribution among males and females. Two patients had known syndromes, 1 with neurofibromatosis type 1 and 1 with von Hippel-Lindau syndrome. All tumors were located in the right adrenal gland and were treated by adrenalectomy. No patients had recurrences or metastases. All were alive and well at follow-up intervals of 1.5 to 8 years (mean, 4.9 years).

The maximum tumor diameter ranged from 1.8 to 5.8 cm with a mean diameter of 3.4 cm. The tumors were well circumscribed and spherical to oval with a gray-tan cut surface. Focal congestion and hemorrhage were present. Microscopically, tumor cells were arranged in nests, trabeculae, and solid sheets, with a predominant Zellballen pattern and a rich vascular stroma. The neuroblastic elements included differentiating neuroblastoma in 1 case, ganglioneuroblastoma in 1 case, ganglioneuroma in 1 case, and mature ganglion cells in 1 case. The case with differentiating neuroblastoma was in the patient with neurofibromatosis type 1 and displayed a neuroblastic component of immature and mature ganglion cells in a schwannian stroma-poor background with abundant neuropil and relatively sparse neuroblasts. The mitotic-karyorrhectic index was low. The ganglioneuroblastomatous and ganglioneuromatous components in 2 patients contained predominantly mature ganglion cells embedded in a schwannian stroma intermingled with classic pheochromocytoma.

Table 1

Clinical and Pathologic Features in Four Cases of Composite Pheochromocytoma

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Clinical Manifestations</th>
<th>Syndrome</th>
<th>Tumor Size (cm)</th>
<th>Morphologic Features</th>
<th>N-myc Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/15</td>
<td>Hypertension</td>
<td>None</td>
<td>3.5</td>
<td>Ganglioneuroblastoma, intermixed, low MKI</td>
<td>Nonamplified</td>
</tr>
<tr>
<td>2/M/17</td>
<td>Adrenal mass</td>
<td>Neurofibromatosis type 1</td>
<td>5.8</td>
<td>Differentiating neuroblastoma, low MKI</td>
<td>Nonamplified</td>
</tr>
<tr>
<td>3/F/32</td>
<td>Adrenal mass</td>
<td>von Hippel-Lindau syndrome</td>
<td>1.8</td>
<td>Ganglion cells in clusters</td>
<td>Nonamplified</td>
</tr>
<tr>
<td>4/F/40</td>
<td>Adrenal mass; idiopathic dilated cardiomyopathy</td>
<td>None</td>
<td>2.5</td>
<td>Ganglioneuroma</td>
<td>Nonamplified</td>
</tr>
</tbody>
</table>

MKI, mitotic-karyorrhectic index.
The tumor with a small component of mature ganglion cells showed typical pheochromocytoma with sparse intermingled mature ganglion cells and no visible schwannian stroma, immature neuroblastic components, or neuropil.

All 4 CPs had absence of N-myc amplification in the neuroblastic components.

**Ordinary Pheochromocytoma**

We selected 10 samples of OP from 9 patients for comparison and evaluation of N-myc amplification. The age range of patients was 9 to 59 years. Of the 9 patients, 5 had known syndromes, including von Hippel-Lindau syndrome, multiple endocrine neoplasia type 2, neurofibromatosis type 1, velocardio-facial syndrome, and an undefined hematoma/polyposis syndrome. The tumor size ranged from 3.1 to 9.0 cm. Of the 9 patients, 2 had recurrences, and all patients were alive and without evidence of recurrence or metastasis at last follow-up.

Of the 10 samples of OP, 9 lacked N-myc amplification, and 1 sample had a minimal elevation of the N-myc/IF-2 ratio at 2.36. Owing to the much larger amplification seen in the neuroblastomas, this minimal elevation was considered equivocal.

**Neuroblastoma**

We selected 10 cases of neuroblastoma to represent a spectrum of undifferentiated, poorly differentiated, and differentiating neuroblastomas with low and high mitotic-karyorrhectic indices for comparison and evaluation of N-myc amplification. The patients ranged in age from 7 months to 6 years at diagnosis, and none had known syndromes. At last follow-up, 5 patients had died of disease and 5 were alive and without evidence of neuroblastoma.

Of 10 neuroblastoma samples, 5 had N-myc amplification that was significantly greater than that seen in CP or OP. Of the 5 patients who died of disease, the samples from 4 showed N-myc amplification.

**Discussion**

Composite tumors of the adrenal medulla are rare and typically consist of a predominant pattern of pheochromocytoma combined with ganglioneuroma, ganglioneuroblastoma, neuroblastoma, or, rarely, other components such as malignant peripheral nerve sheath tumor and neuroendocrine carcinoma. The frequency of composite adrenal tumors has been reported as ranging from less than 3% of all adrenal gland neoplasms to between 1% and 9% of pheochromocytomas. Although it is recognized that CP can be associated with genetic disorders such as neurofibromatosis type 1, von Hippel-Lindau disease, and multiple endocrine neoplasia, similar to the conditions associated with OP, specific pathologic-prognostic or genetic-prognostic factors have not been identified. Little information is available about the outcome of CP because of its rarity. Some reports have described indolent behavior. However, metastases have been reported in CP with ganglioneuroblastoma and ganglioneuroblastoma. Late recurrence of CP with ganglioneuroblastoma has also been reported. We report the clinicopathologic features, outcome, and results of N-myc analysis for CP and the comparison with selected cases of OP and neuroblastoma. The 4 CPs harbored favorable histologic neuroblastic elements with a low mitotic-karyorrhectic index. All patients with CP were alive and had no evidence of recurrence or metastasis at intervals ranging from 1.5 to 8 years. All cases showed absence of N-myc amplification with polymerase chain reaction analysis. Similar to the CPs, the 10 selected cases of OP lacked N-myc amplification and had a favorable outcome. The 10 cases of neuroblastoma were selected to include a spectrum of neuroblastic tumor morphologic features and mitotic-karyorrhectic indices ranging from low to high. Of the neuroblastomas, 5 had N-myc amplification that was significantly greater than the values seen in CP and OP, and 4 of the 5 patients had died of disease at last follow-up.

The rarity of CP and the paucity of information about its biologic potential and outcome in the literature, apart from CP with malignant peripheral nerve sheath tumor, has hampered...
Image II Composite pheochromocytomas. A and B, Nodules of maturing ganglion cells with neuropil in a background of typical pheochromocytoma and mild hemorrhage (A, H&E, ×100; B, H&E, ×200). C, Mature ganglion cells (H&E, ×400). D, Maturing neuroblastoma with extensive hemorrhage (H&E, ×400). E and F, Mature ganglion cells with mild hemorrhage (E, H&E, ×100; F, H&E, ×200).
the understanding of the clinical and therapeutic significance of neuroblastic elements in CP. Although a previous report suggested that recognition of neuroblastomatous foci in CP might be clinically important, there are few data in the literature to address this question. On the other hand, biologic and pathologic predictors of outcome in neuroblastic tumors have been studied extensively during the past several decades. It is well recognized that the presence of N-myc amplification is an unfavorable prognostic feature in neuroblastoma. Other unfavorable prognostic indicators for neuroblastic tumors include age and stage at diagnosis, histologic subtype, mitotic-karyorrhectic index, and a variety of other cytogenetic and molecular genetic features. The 4 CPs that we report all had favorable histologic-prognostic features and lacked N-myc amplification, and all patients had a favorable outcome. Other reports of neuroblastic components in CP have emphasized indolent behavior with a favorable outcome or have not provided outcome information. Genetic analyses in CP have concentrated on NFI and RET mutations, without identifying prognostically significant features. Occasionally patients with CP have symptoms related to vasoactive intestinal polypeptide production or elevated catecholamine levels, with varying degrees of diarrhea and hypertension.

This study demonstrates that neither CP nor classic pheochromocytoma harbors N-myc amplification. The neuroblastic elements in CP recapitulate favorable histologic neuroblastoma in their pathologic features, low mitotic-karyorrhectic index, absence of N-myc amplification, and favorable outcome. These results suggest that CP does not have adverse prognostic significance conferred by the neuroblastic elements. The main limitation of the present study is the small number of cases of CP available for examination, with only 1 case having immature neuroblastic elements. Also, while we confined the genetic analysis of tumor tissue to evaluation for N-myc amplification, it is possible that there might be other genetic or biologic markers for aggressive behavior in CPs that are not identifiable with the current methods. The CPs in this study had favorable neuroblastic elements; if a CP with unfavorable neuroblastic elements were seen, N-myc analysis may be helpful in assessing prognosis and outcome and guiding clinical management. Further studies of these extraordinarily rare tumors, including those with unfavorable histology neuroblastic elements and those with more aggressive clinical courses, are needed to more comprehensively evaluate whether there are any identifiable and significant prognostic, therapeutic, or biologic differences between OP and CP.

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References