Expression of Extracellular Signal-regulated Kinase 5 and Ankyrin Repeat Domain 1 in Composite Pheochromocytoma and Ganglioneuroblastoma Detected Incidentally in the Adult Adrenal Gland

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Abstract

Composite pheochromocytoma (cPC) is extremely rare, arising in the adrenal medulla as a mixture of PC and other tumors of neural origin. We herein report on a case of adrenal incidentaloma post-operatively diagnosed as cPC with ganglioneuroblastoma (GNBL). The PC component had 7 points on the PASS, a Ki-67 index of 5.1%, a focal absence of sustentacular cells, and no genetic aberrations in succinate dehydrogenase subunit B. The GNBL component exhibited no N-myc amplification. Tumor cells of both components were stained positively for extracellular signal-regulated kinase 5 and ankyrin repeat domain 1. The aberrant activation of growth signaling may play a role in the marginal malignancy of cPC.

Key words: adrenal, composite pheochromocytoma, ganglioneuroblastoma, extracellular signal-regulated kinase 5, ankyrin repeat domain 1


Introduction

Composite pheochromocytoma (PC) is a rare neoplasm that typically arises in the adrenal medulla as a mixture of PC and other tumors of neural origin, including peripheral neuroblastic tumors, malignant peripheral nerve sheath tumors, and neuroendocrine carcinomas (1, 2). The frequency of composite PCs in the adrenal gland has been estimated to be <10% of the total number of adrenal PCs (3, 4). To the best of our knowledge, approximately 75 adrenal (1, 2, 5-15) and 12 extra-adrenal (16-24) cases have been reported in the medical literature to date.

Peripheral neuroblastic tumors encompass a wide spectrum of differentiation and malignancy potentials from “the most primitive and malignant” neuroblastomas (NBLs) to “the most mature and benign” ganglioneuromas (GNs). Ganglioneuroblastomas (GNBLs) are an intermediate stage of neuroblastic tumors (25, 26). GNs coexist most frequently in 72.6% and 92.9% of adrenal (1, 2, 5-15) and extra-adrenal (16-24) composite PCs, respectively. However, other types of composite PCs are extremely rare, with only a few cases having been reported for adrenal PC-GNBLs (2, 25, 27-37).

Very recently, we revealed for the first time that extracellular signal-regulated kinase 5 (ERK5) together with ankyrin repeat domain 1 (ankrd1) induced by ERK5 regulates tyrosine hydroxylase (TH) activity and catecholamine biosynthesis during neural differentiation, with ERK5 expression levels correlated with TH levels in the normal adrenal medulla (38). However, ERK5 was down-regulated, and the ERK5-mediated regulation of the cellular function was disrupted in human adrenal PC (38).

Following our review of the previous literature, we herein...
report on a rare case of adrenal incidentaloma in a normotensive patient who was diagnosed post-operatively with a composite PC, occurring together with a GNBL component from the right adrenal medulla on microscopic findings. In addition, we also immunohistochemically investigate the ERK5 regulation in composite adrenal PC-GNBL.

**Case Report**

**Clinical course**

A 55-year-old Japanese woman who was receiving medication for hyperlipidemia at the discretion of an office physician had a right adrenal tumor (18 mm in diameter) that was detected incidentally by plain computed tomography (CT) imaging of the chest during the examination of a lung bulla. Despite the physician’s recommendation, the patient failed to attend further check-ups of the adrenal tumor, as she experienced no further symptoms and had a tight business schedule.

Five years later, at 60 years of age, the right adrenal tumor had increased in diameter to 30 mm on a follow-up CT scan when the patient was referred to our hospital for further evaluation of the adrenal mass. The patient had not complained of sudden headaches, palpitations or sweating and had no history of hypertension, constipation, or diarrhea. Here comorbidities included hyperlipidemia and pulmonary emphysema resulting from a smoking habit of 24 years. The patient’s medical history included rheumatic fever in her childhood and subacute thyroiditis at the age of 40. No family history of inherited genetic syndromes (e.g., multiple endocrine neoplasms, neurofibromatosis or von Hippel-Lindau disease) was reported.

A physical examination showed the patient’s height, weight, blood pressure, and heart rate to be 154.5 cm, 59.0 kg, 138/84 mmHg, and a regular 82 beats/min, respectively. Her blood cell counts, biochemical data, and urinalysis findings were all within the reference limits, except for an overproduction of catecholamine (Table 1). The 24-hour urinary excretion of adrenaline (96.8 μg/d), dopamine (1,269.5 μg/d), metanephrine (1.2 mg/d), and normetanephrine (0.54 mg/d) was increased. Blood noradrenaline levels (1.05 ng/mL) and urinary noradrenaline (181.1 μg/d) were slightly above the upper reference limit.

Noncontrast CT clearly demonstrated a mass of a heterogeneous nature within the right adrenal gland. The tumor was strongly and inhomogeneously enhanced with contrast medium on CT. Magnetic resonance imaging (MRI) of the tumor revealed low and high signal intensities on T1- and T2-weighted images, respectively. Gadolinium-enhanced T1-weighted imaging of the abdominal MRI demonstrated the presence of an irregularly shaped low-signal-intensity region within the tumor, partially surrounded by well-enhanced regions with clear demarcation (Fig. 1). Scintigraphy of the tumor revealed strong accumulations of 123I-metaiodobenzylguanidine. The patient was diagnosed clinically with a right adrenal PC and underwent laparoscopic right adrenalectomy (operation time: 2 hours 55 minutes, blood loss: 5 mL). The intra-operative and post-operative course was uneventful, with no abnormal fluctuations in blood pressure or heart rate. Adjuvant treatment was not administered, and no tumor recurrence was detected 17 months after surgery.

**Pathological findings**

On gross appearance, the cut surface of the resected specimen exhibited a heterogeneous, irregularly lobulated, dark-brownish area in the central region that was accompanied by focal hemorrhage. Distinct solid and white regions dominated the periphery outside the brown-colored area in the central tumor (Fig. 1). The morphology of the cut section was consistent with the T2-weighted and gadolinium-enhanced T1-weighted MRI findings observed before surgical resection (Fig. 1).

Microscopically, the tumor consisted of two different components: PC and GNBL (Fig. 2A-C). The PC component was located within the dark, inhomogeneous region of the central tumor, while the GNBL component existed outside of the PC region at the periphery of the tumor (Fig. 1D and E). The PC component demonstrated histological features typical of PC, consisting of polygonal tumor cells with granular and basophilic cytoplasm, round-to-oval nuclei, and a single prominent nucleolus, distributed in well-separated nests with thin stroma and rich vascularity (Zellballen pattern) (Fig. 2A and 3A and the inset).

Our immunohistochemical analyses demonstrated that the PC component stained diffusely positive for chromogranin A (Fig. 2B) and synaptophysin (Supplementary file 1) in the

### Table 1. Catecholamines and Their Metabolites.

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<td>1,269.5 μg/d</td>
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<td>0.54 mg/d</td>
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<tr>
<td>homovanillic acid</td>
<td>4.92 mg/d</td>
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Figure 1. The clinicopathological appearance of the adrenal composite pheochromocytoma. Computed tomography revealed a right adrenal tumor which had a heterogeneous internal structure on a plain image (A) and was inhomogeneously enhanced with contrast medium (B). The tumor exhibited high signal intensities on T2-weighted magnetic resonance imaging (C). On gadolinium-enhanced T1-weighted imaging, the tumor also presented as an irregular-shaped low-signal-intensity area partially surrounded by strongly enhanced outer regions (D). On gross appearance, the cut surface of the resected tumor consisted of two different components: a brown component in the central region and a whitish component at the periphery (E), consistent with radiological images (A-D). Similarly, the two components were readily discernible on the tumor sections prepared for microscopic observation using Hematoxylin and Eosin staining (F). Of note, panel F was synthesized intentionally by combining two photos of different pathological preparations with reference to the tumor morphology in panels A-E.

tumor cells and stained positive for S-100 protein (Fig. 2C, 3D) in the surrounding sustentacular cells. The tumor cells proliferated in diffuse patterns and invaded neighboring blood vessels (Fig. 3C). Disappearance of the sustentacular cells was partially observed in the PC region (Fig. 3D). The Ki-67 proliferative index was noted to be at...
5.1% in the highest of the PC regions (Fig. 3E and the inset). High cellularity, cellular monotony, and tumor cell spindling were also observed (Fig. 3B), although mitotic figures and necrosis were absent (Fig. 3A). These findings suggested a malignant potential, yielding a Pheochromocytoma of the Adrenal gland Scaled Score (PASS) of 7 points (39).

In the present case, the PC component was found not to express vasoactive intestinal peptide (Fig. 3F) but was, however, positively immunostained for antibodies against succinate dehydrogenase subunit B (SDHB), suggesting a sporadic nature with no mutations in the SDHB gene (Fig. 4).

In contrast, the GNBL component comprised a mixture of variably differentiated neuroblastic cells and ganglion-like cells in a neurofibrillary background, with no primitive neuroblastic foci (Fig. 2A and D). The GNBL component stained positively for anti-S-100 protein (Fig. 2C), neurofilament, and synaptophysin antibodies (Supplementary file 1), presenting with a characteristic morphology of GNBL-intermixed, according to the international neuroblastoma pathology classification (26).

Fig. 5 shows the immunohistochemical expression of TH, ERK5, and ankrd1 protein in both PC and GNBL components in the composite adrenal tumor. TH, a key enzyme which produces catecholamines, was expressed diffusely in the PC and neuroblastic cells. However, ERK5- and ankrd1-positive tumor cells were scattered over both of the components in the composite tumor. These findings were consistent with our recent findings regarding human adrenal PC (38), suggesting that TH may be regulated not only by an ERK5/ankrd1 signaling cascade but also aberrantly by other unknown mechanisms.

The immunohistochemical staining methods (38, 40) and fluorescence in-situ hybridization analyses used in the present report are described in detail in the supplementary file (Supplementary file 2).

Cytogenic analysis of the N-myc gene in ganglioneuroblastomas using fluorescence in-situ hybridization

N-myc gene status was evaluated via interphase fluorescence in-situ hybridization in 3-μm-thick tissue sections from a paraffin-embedded formalin-fixed surgically resected clinical specimen, using previously described experimental protocols (41) and Supplementary file 2. Fluorescence in-situ hybridization signals were scored in 200 non-overlapping tumor nuclei. In the present case (Fig. 6), dip-
loidy patterns [2 N-myc and 2 centrosomal protein 2 (CEP2) signals] were identified in 169 tumor cells (84.5%). A further 5 cells (2.5%) exhibited 0 to 1 N-myc and 2 CEP2 signals, and 21 cells (10.5%) displayed a hyperploidy pattern (3 to 5 N-myc and 3 to 5 CEP2 signals) in the nuclei. Copy number gain of the N-myc gene, 1 copy greater than the CEP2 probe signals (3 N-myc and 2 CEP2 signals), was detected in 5 GNBL cells (2.5%) (Fig. 6, arrows and arrow

**Figure 3.** The microscopic features of the pheochromocytoma (PC) component of the right adrenal composite PC. Focal hemorrhaging was observed in the PC component, but no confluent necrosis or mitosis was observed on Hematoxylin and Eosin (H&E) staining (A). The PC component comprised two types of tumor cells: large polygonal eosinophilic cells with pleomorphic nuclei and occasionally prominent nucleoli and coarse chromaffin (left half of inset A); and small, round chromaffin cells (right half of inset A). Both types of cells were distributed in the form of variably sized nests, called Zellballen structures (A). There were also sheet-like areas and spindle cell arrangements on H&E staining (B). Although high cellularity, cellular monotony, and tumor cell spindling were noted, neither mitosis nor necrosis was evident (A, B). There was focal vascular invasion in the PC component on Elastica-Masson’s staining (C). The sustentacular cells, stained positively for S-100, partially disappeared in the PC region where the Zellballen structures were poorly aligned (D). In (D), the arrowheads indicate the sparse localization of the sustentacular cells around the disordered Zellballen nests. The PC cells were positive for the Ki-67 antigen at a maximum of 5.1% (E and the inset) but did not show any expression of vasoactive intestinal peptide (F) (original magnification 4× for A, 200× for B, 400× for C, and 100× for D-F; the insets of A and E were originally magnified at 400×).
Figure 4. The succinate dehydrogenase subunit B immunohistochemical analysis of the adrenal composite pheochromocytoma (PC). The two components constituting the adrenal composite PC expressed succinate dehydrogenase subunit B as detected by immunohistochemistry. The right half of the image represents the PC component, while the left half represents the ganglioneuroblastoma component of the composite PC (original magnification 200×).

Discussion

Composite PCs are exceedingly rare tumors - particularly composite PC-GNBL tumors, where fewer than 20 cases (2, 25, 27-36), including the present, having been reported in the medical literature to date (Table 2).

Shawa et al. (6) described how composite PCs could not be distinguished from pure PCs based on clinical presentations, biochemical data, and CT images alone, owing to similar symptomatology, biochemical results, morphology, and attenuation values. Additionally, no reports on the differential radiographic features between composite and pure PCs have been published. Although up to 70% of PCs present with high signal intensities on T2-weighted MRI (designated as the light bulb sign), at least 30% of PCs exhibit moderate or low T2-weighted signal intensities on MRI and appear similar to other adrenal diseases (42). The findings on ultrasonography, CT scans, MRI, 123 I-metaiodobenzylguanidine scintigraphy, and positron emission tomography complement each other with respect to differentiating PCs from other adrenal diseases in clinical practice (42-44). Furthermore, it is also impossible to clinically differentiate between NBLS, GNBLs, and GNs, owing to variable appearances on imaging analyses; although GNs tend to be more homogeneous (45). Most case reports on composite PCs have determined a diagnosis based on the post-operative histopathological observations (1), except for two cases in which the authors performed pre-operative and intra-operative biopsies (46, 47). Thus, we believe that it is not possible to predict clearly whether PCs are pure or composite or to determine what neural elements would be combined with PCs if pathological exploration was not completed.

It is widely accepted that the biological behavior of PCs cannot be predicted on the basis of histopathological features alone (39). The diagnosis of a malignant PC can be determined by the presence of recurrence or a metastatic lesion at a site where chromaffin cells are not normally present (4). Although around 10% of pure PCs have been considered malignant, many more cases may become metastatic over the long duration of follow-up after the initial diagnosis. Recently, Ayala-Ramirez et al. (48) demonstrated that 25% and 60% of 267 and 104 patients with adrenal and extra-adrenal PCs had metastatic diseases, with an overall survival of 20.64 or 9.49-years, respectively. As for composite PCs, Khan et al. (1) documented that approximately 25% of total composite PCs had metastasized during the observation period. Composite PCs can metastasize from either the sympathetic or the neural components, both of which are generally thought to be derived from common chromaffin precursor cells by aberrant differentiation (1). Metastatic elements of composite PCs from its primary site vary on a case by case basis. Some involve PC cells (3, 6), while others are neural (15, 20, 27) or involve both components (49).

Among the neural elements in composite PCs, neuroblastic tumors coexist most frequently. Generally, neuroblastic tumors can be classified into three pathological disease conditions according to the extent of differentiation, as NBLS, GNBLs, or GNs if the tumors are poorly, intermediately, or well-differentiated, respectively. NBLS are malignant tumors consisting of primitive neuroblasts that may arise anywhere within the sympathetic plexus or adrenal medulla (50). NBLS are considered more aggressive than GNs, which usually behave in a benign fashion (50). GNBLs belong to an intermediate group between NBLS and GNs, both in terms of maturation and malignancy potential (45). NBLS and GNBLs are typically pediatric diseases (26, 50).

The prognosis of patients diagnosed with neuroblastic tumors depends on factors such as age at diagnosis, tumor grading and stage, N-myc amplification status, and deletion of chromosome 1p (26, 51). In approximately 20% of NBLS the human proto-oncogene N-myc is amplified, the presence of which is generally indicative of a poor prognosis for NBLS (2, 26). N-myc amplification was found to occur only in around 2% to 3.1% and 0% of 32 GNBLs and 10 GNs, respectively (41, 52). In contrast, adult-onset NBLS and GNBLs are extremely rare, with fewer than 100 cases of these tumors having been reported in adults in the English medical literature to date (53, 54). In comparison to pediatric cases, N-myc is less frequently amplified in adult-onset cases (53). In several recent reviews (53, 54), between 35% and 50% of adult-onset cases have reportedly presented with metastases at diagnosis, seemingly suggesting a relatively poor oncological outcome. At present, however, the tumor biology and clinical prognoses remain unclear, due to disease rarity, and so treatment guidelines on adult-onset NBLS or GNBLs have not yet been established, with clinical prac-
Khan et al. (1) described distant metastasis as having occurred in 1 of 30 adrenal composite PC-GN tumors and 9 of 11 cases with adrenal composite PC mixed with an NBL, malignant peripheral nerve sheath tumor, or neuroendocrine carcinoma. These findings suggest that the tumor behavior and prognosis of composite PCs are greatly influenced by the type of neural components involved. According to Wang et al. (40), copy number gain (1 to 9 copies more than CEP2 gene) and amplification (at least 10 copies greater than those) of the \textit{N-myc} gene were observed in 78.1% and 3.1% of 32 patients with GNBLs and NBLs, respectively. They also demonstrated that \textit{N-myc} gain was a favorable prognostic factor in 220 pediatric patients with neuroblastic tumors, compared with a normal and amplified status of the \textit{N-myc} gene. In addition, hyperploidy was also found to be associated with a favorable prognosis in neuroblastic tumors (55, 56). In the present case, the gene status of \textit{N-myc} in the GNBL component was not amplified but gained. Furthermore, 10.5% of GNBL cells presented with hyperploidy. These findings support the benign nature of the neuroblastic component of the present composite PC. To the best of our knowledge, the presence of amplified \textit{N-myc} has not yet been confirmed in the neural elements of composite PCs (2, 20). Comstock et al. (2) have insisted that compos-
ite PCs should be regarded as a histological variant of pure PCs rather than a separate disease entity.

Strong et al. (4) reported that all malignant PCs had a PASS ≥6 points and recommended that patients with a PASS ≥4 points should be monitored closely for recurrence. de Wailly et al. (57) recently suggested the presence of tumor necrosis, a Ki-67 proliferative index of ≥4%, and the absence of S-100 protein as new criteria for recurrence and a high risk of malignancy. It has also been revealed that the incidence of malignancy in PCs is much greater with SDHB mutations than without (58). A deletion of the SDHB gene has recently been associated with composite paraganglioma with NBL (59). In the present case, the PC component was stained positively for SDHB but had histological features consistent with a PASS of 7 points, a Ki-67 proliferative index of 5.1%, and the focal absence of sustentacular cells staining positive for S-100 protein on immunohistochemical analysis. These findings suggested that the PC component of the present case of composite PC might be malignant but lacking any clinically detectable metastases at the current stage.

In our previous report (37), ERK5 participated in neuronal growth and catecholamine biosynthesis, and ankrd1, a target molecule of ERK5, regulated TH in an ERK5-dependent manner. Unlike normal chromaffin cells in the adrenal medulla, ERK5 regulation of catecholamine levels is disrupted in human PCs (37), which may be because some cases of PC show few sympathetic symptoms associated

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**Table 2. Reported Cases of Composite Pheochromocytoma (PC) with Ganglioneuroblastoma (GNBL).**

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with catecholamine overproduction while others behave marginally in malignancy, as a consequence of the aberrant role of ERK5 in tumor biology. Further follow-up of the present case and examination of more cases of composite PCs is therefore necessary to clarify whether the tumor itself is benign or malignant.

We herein reported on a very rare case of adrenal composite PC concomitant with GNBL that was incidentally detected and clinically asymptomatic, despite catecholamine overproduction, and had not been metastatic or recurrent in the approximate 6.5 years since initial diagnosis. The PC component of the tumor demonstrated pathological characteristics consistent with a PASS of 7 points, a maximum Ki-67 proliferative index of 5.1%, the focal absence of sustentacular cells, and a lack of any genetic aberration in the SDHB gene. The GNBL component of the tumor showed DNA hyperploidy but no amplification of N-myc. Tumor cells of the PC and GNBL components expressed ERK5 and ankr1 protein variably cell by cell, inconsistent with diffuse expression of TH, a key enzyme which produces catecholamines. These findings suggest that the ERK5/ankr1 signaling cascade may aberrantly play more roles in tumor growth in composite PCs than in the normal adrenal medulla. Our findings support the potentially malignant nature of the PC component in the present case of composite PC-GNBL. However, further long-term follow-up and large-scale studies of this rare disease will be needed to more definitively evaluate the oncological outcome.

**Consent**

Written informed consent was obtained from the patient for publication of this case report, any accompanying images, the clinical data, and the results of the tumor genetics analyses. A copy of the written consent form is available for review by the editor of this journal.

The present study was performed in accordance with the principles embodied in the Declaration of Helsinki and was approved by the Ethical Committee of Yamagata University Faculty of Medicine (H25-98 and H27-122).

**The authors state that they have no Conflict of Interest (COI).**

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**References**


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