INTERNAL MEDICINE

□ CASE REPORT □

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

(Intern Med 55: 3611-3621, 2016)
(DOI: 10.2169/internalmedicine.55.7293)

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

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Received for publication February 19, 2016; Accepted for publication April 3, 2016
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Table 1. Catecholamines and Their Metabolites.

Specimens		Concentrations		Reference ranges			
Plasma	adrenaline	0.18	ng/mL	0	–	0.10	ng/mL
	noradrenaline	1.05	ng/mL	0.06	–	0.46	ng/mL
	dopamine	0.05	ng/mL	0	–	0.02	ng/mL
24 h-urine	adrenaline	96.8	μg/d	3.4	–	26.9	μg/d
	noradrenaline	181.1	μg/d	48.6	–	168.4	μg/d
	dopamine	1,269.5	μg/d	65.0	–	961.5	μg/d
	metanephrine	1.2	mg/d	0.04	–	0.19	mg/d
	normetanephrine	0.54	mg/d	0.09	–	0.33	mg/d
	vanilylmandelic acid	4.71	mg/d	2.2	–	6.0	mg/d
	homovanillic acid	4.92	mg/d	1.5	–	4.9	mg/d

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 ^{123}I-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

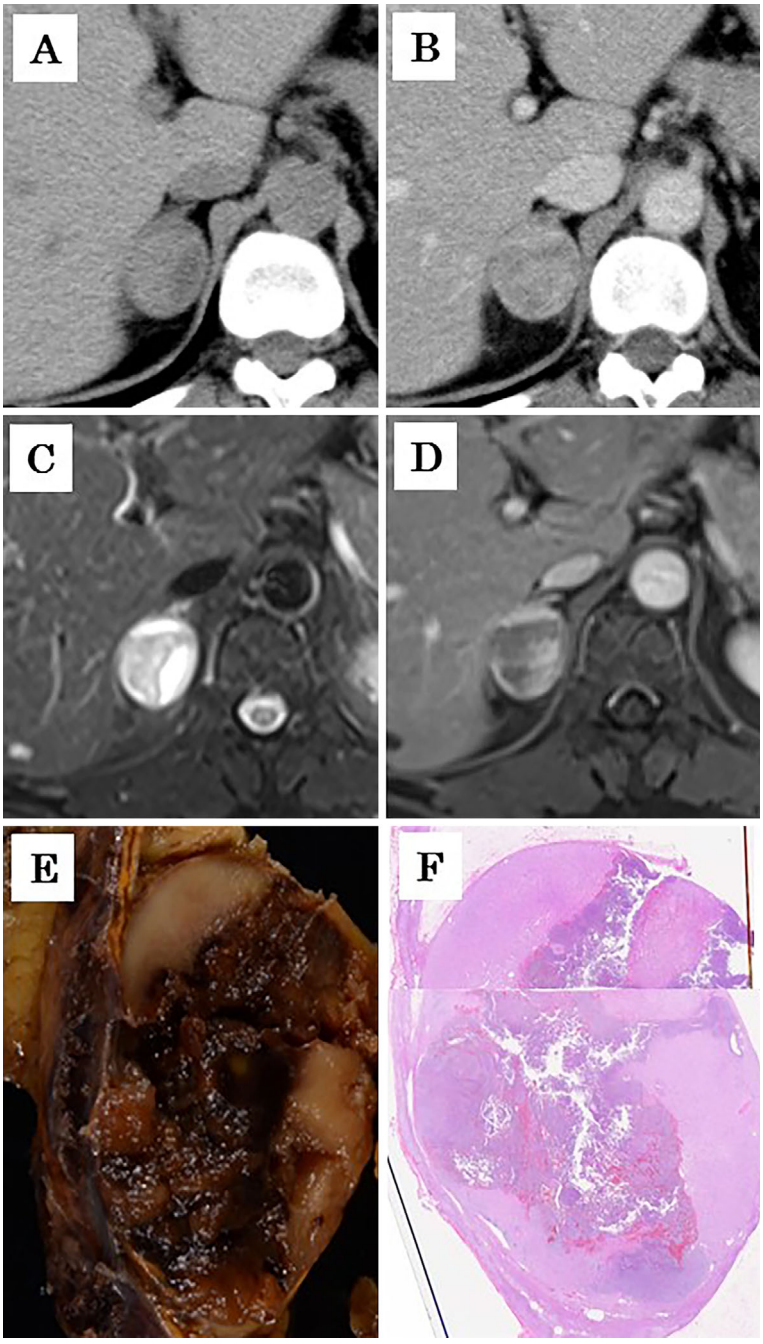

Figure 1. **The clinicopathological appearance of the adrenal composite pheochromocytoma. Computed tomography revealed a right adrenal tumor which had a heterogeneous internal structure on a plain image (A) and was inhomogeneously enhanced with contrast medium (B). The tumor exhibited high signal intensities 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 discernable 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

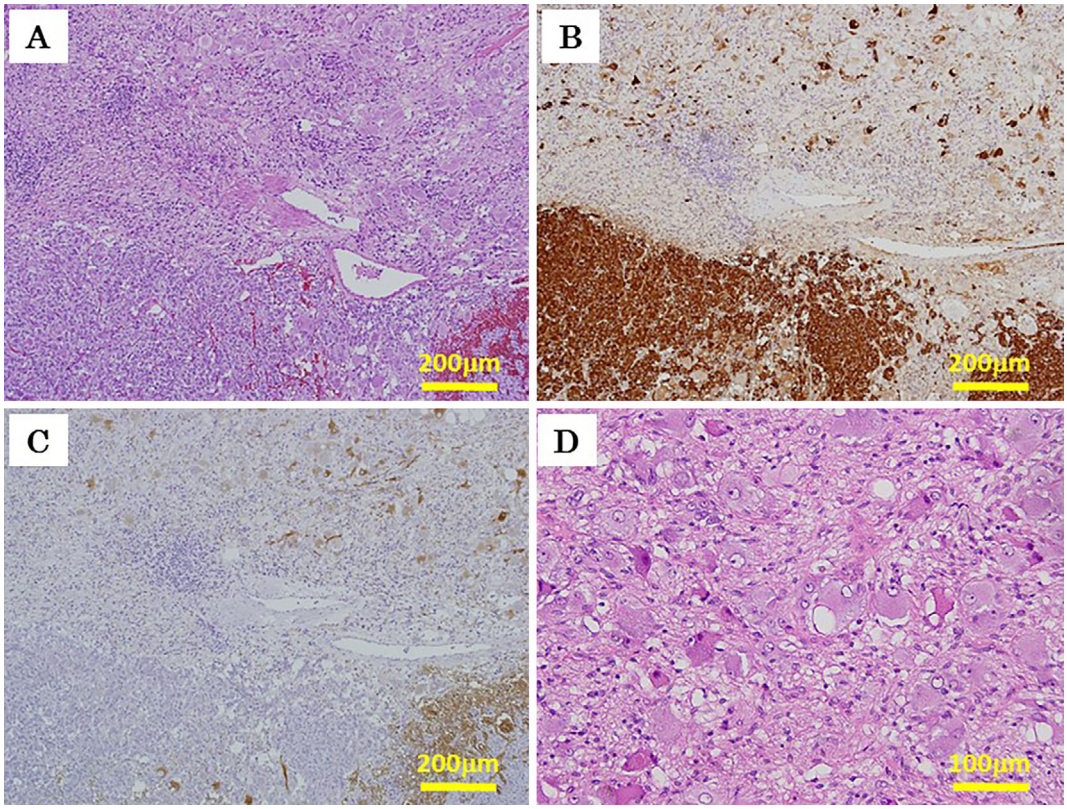

Figure 2. **Pathological microphotographs of the right adrenal composite pheochromocytoma with ganglioneuroblastoma. Different sections at the same position of the tumor were stained with Hematoxylin and Eosin staining (A) and for chromogranin A (B) and S-100 protein (C). The upper and lower halves of panels A to C represent the ganglioneuroblastoma and pheochromocytoma components of the tumor, respectively. In panel D, the GNBL component comprised a mixture of ganglion-like cells in a neurofibrillary background with no primitive neuroblastic foci, presenting with a characteristic morphology of GNBL-intermixed according to the international neuroblastoma pathology classification (original magnification 100× for A, B, and C, 200× for D).**

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-

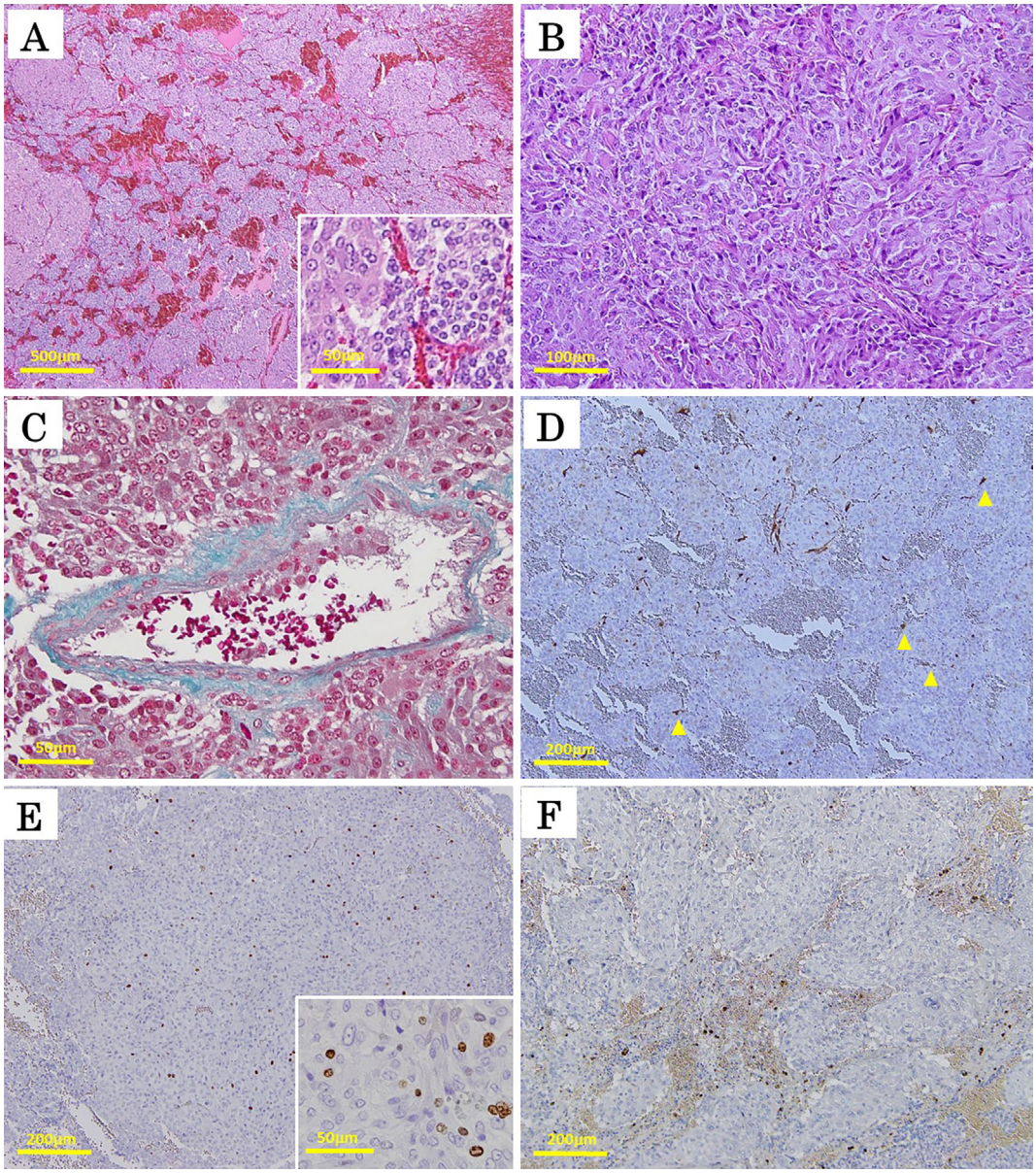

Figure 3. **The microscopic features of the pheochromocytoma (PC) component of the right adrenal composite PC. Focal hemorrhaging was observed in the PC component, but no confluent necrosis or mitosis was observed on Hematoxylin and Eosin (H&E) staining (A). The PC component comprised two types of tumor 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×).**

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

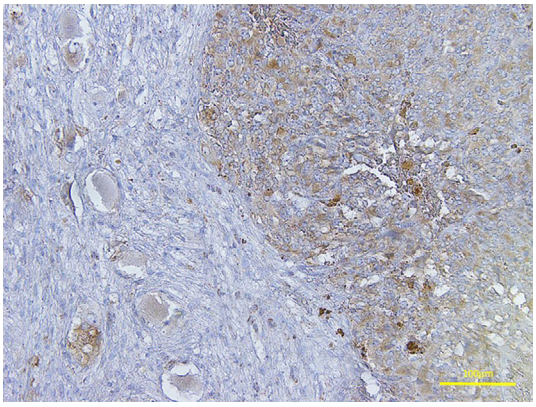

Figure 4. The succinate dehydrogenase subunit B immunohistochemical analysis of the adrenal composite pheochromocytoma (PC). The two components constituting the adrenal composite PC expressed succinate dehydrogenase subunit B as detected by immunohistochemistry. The right half of the image represents the PC component, while the left half represents the ganglioneuroblastoma component of the composite PC (original magnification 200×).

heads). None of the tumor cells demonstrated *N-myc* amplification (at least 10 copies greater than the CEP2 gene).

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 is dependent 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-

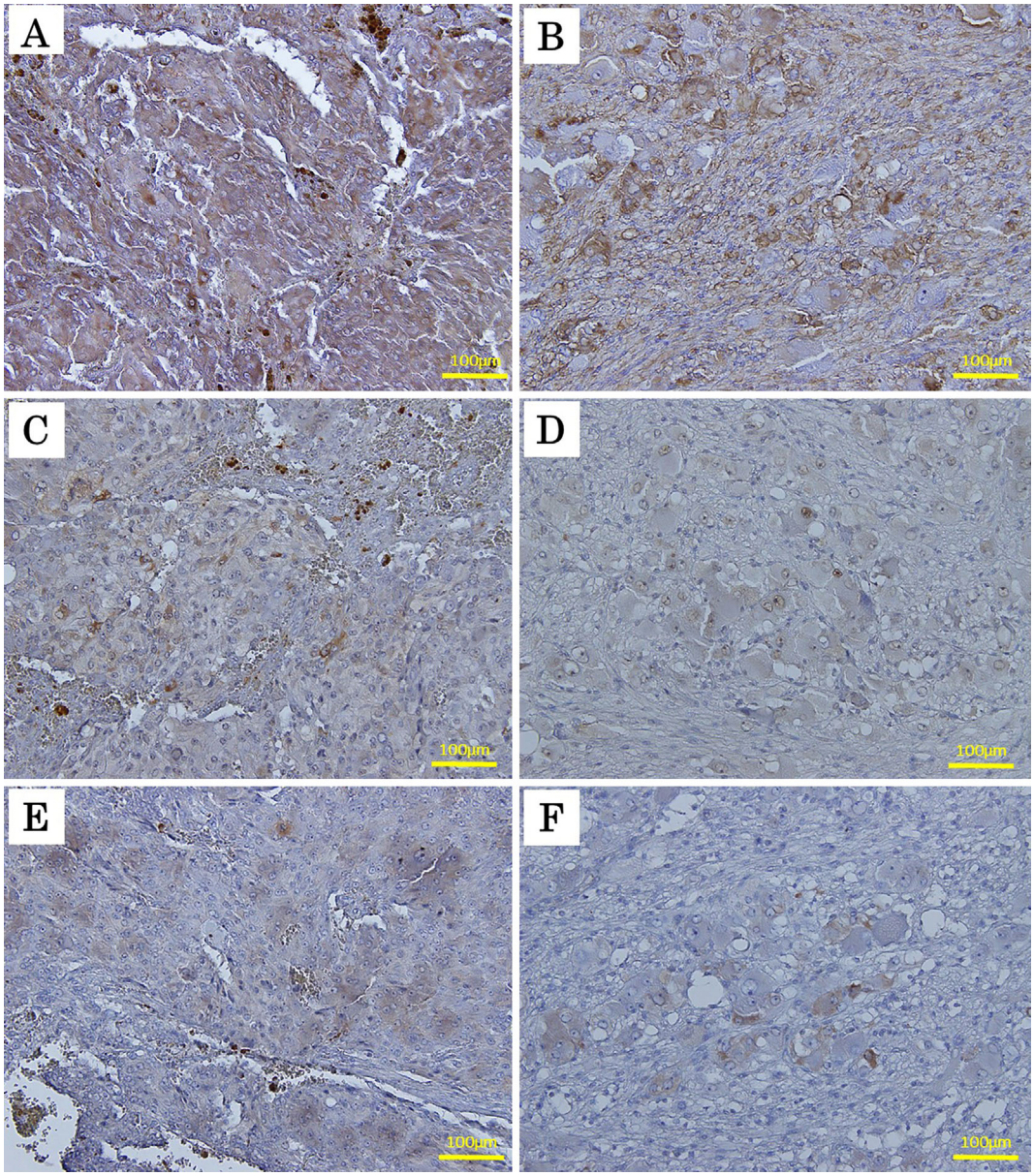

Figure 5. **The immunohistochemical findings for thyrosine hydroxylase (TH), extracellular signal-regulated kinase 5 (ERK5), and ankyrin repeat domain 1 (ankrd1) protein in the pheochromocytoma (PC) and ganglioneuroblastoma (GNBL) components in the composite adrenal tumor. Both the PC and GNBL components diffusely expressed TH (A and B, respectively), but parts of the tumor cells had positive staining for anti-ERK5 (C and D, respectively) and ankrd1 (E and F, respectively) antibodies in both components (original magnification 200×).**

tice regimens only able to be extrapolated from the pediatric guidelines (26, 53, 54).

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 *N-myc* gene were observed in 78.1% and 3.1% of 32 patients with GNBLs and NBLs, respectively. They also demonstrated that *N-myc* gain was a favorable prognostic factor in 220 pediatric patients with neuroblastic tumors, compared with a normal and amplified status of the *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 *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 *N-myc* has not yet been confirmed in the neural elements of composite PCs (2, 20). Comstock et al. (2) have insisted that compos-

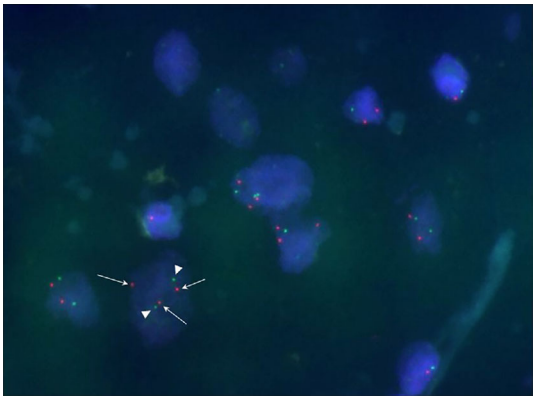

Figure 6. **Fluorescence *in-situ* hybridization image of ganglioneuroblastoma cells displaying gain of N-myc gene copies. The fluorescence *in-situ* hybridization signals were scored in 200 non-overlapping tumor nuclei. Red and green fluorescence signals indicated N-myc and centrosomal protein 2 (CEP2) gene copies, respectively. Tumor nuclei were counterstained with 4',6-diamidino-2-phenylindole. Normal ploidy patterns (2 N-myc and 2 CEP2 signals) were detected in 169 tumor cells (84.5%). In 5 cells (2.5%), the number of N-myc signals (arrows) was 1 copy greater than the CEP2 probe signals (3 N-myc and 2 CEP2 signals, arrow heads). A further 5 cells (2.5%) displayed 0 to 1 N-myc and 2 CEP2 signals, and 21 cells (10.5%) exhibited a polyploidy pattern (3 to 5 N-myc and 3 to 5 CEP2 signals) in the nuclei (original magnification 1,000×).**

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

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

Case	References	Age (years)	Sex	Location	Affected side	Genetic disorder	Hyper-tension	*N-myc* status in GNBL	DNA ploidy pattern in GNBL	Malignancy	Metastasis	Follow-up	Prognosis
1	28	42	F	adrenal	ND		–			–	–		alive
2	27	14	F	adrenal	Rt	NF type-1	–	NA	NA	malignant PC	liver, lung, lymph nodes and bones	6 mo	dead
										PC + GN (primary)			
3	29	43	M	adrenal	Rt	–	–	NA	NA	malignant PC + GNBL (reccurence)	liver	10 yrs	dead
4	30	25	M	adrenal	Rt	–	Yes	NA	NA	–	–	ND	alive
5	31	63	F	adrenal	Rt	–	Yes	NA	NA	malignant PC	liver	5 mo	dead
6	32	21	F	retroperitoneal	ND	–	Yes	NA	NA	–	–	ND	alive
7	33	35	M	adrenal	Rt	NF type-1	Yes	NA	NA	–	–	ND	ND
8	33	42	M	adrenal	Lt	–	Yes	NA	NA	–	–	ND	ND
9	34	49	M	adrenal	Lt	MEN 2A	Yes	NA	NA	thyroid medullary carcinoma	–	–	dead
10	35	29	F	adrenal	Lt	–	Yes	NA	NA	–	–	5 yrs	alive
11	36	55	F	adrenal	Rt	–	–	NA	NA	–	–	11 mo	alive
											lung SCC metastasis		
12	37	73	M	adrenal	Lt	–	–	NA	NA	lung SCC	to the left adrenal	ND	ND
13	2	15	M	ND	Rt	–	Yes	no amp.	NA	–	–	ND	alive
14	25	9	F	adrenal	Rt	–	Yes	no amp.	diploidy	–	–	18 mo	alive
15	Current case	60	F	adrenal	Rt	–	–	no amp.	hyperploidy	–	–	6.5 yrs*	alive

Abbreviations: amp: amplification, F: female, GN: ganglioneuroma, GNBL: ganglioneuroblastoma, Lt: left, M: male, MEN: multiple endocrine neoplasm, mo: months, NA: not accessed, ND: not described, NF: neurofibromatosis, PC: pheochromocytoma, Rt: right, SCC: squamous cell carcinoma, yrs: years, –: no

*: Follow-up duration after initial presentation (19 months since surgical resection).

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 ankrd1 protein variably cell by cell, inconsistent with diffuse expression of TH, a key enzyme which produces catecholamines. These findings suggest that the ERK5/ankrd1 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).

Acknowledgement

This work was supported in part by Grants-in-Aid from the Japan Society for the Promotion of Science (No. 15K07963 to Y. O), and Takeda Science Foundation (Y.O). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

We greatly appreciate the excellent technical support of Dr. Naing Ye Aung from the Institute for Promotion of Medical Science Research at Yamagata University Faculty of Medicine. We thank assistant professors Atsushi Yamagishi, Tomohiro Shibasaki and Hisashi Kawazoe from the Department of Urology at Yamagata University Faculty of Medicine for their clinical cooperation. We also thank Kumiko Hayashi and Takeshi Nakamura from Chromosome Analysis Group, Molecular Genetic Analysis Department, LSI Medience Corporation (Tokyo, Japan) for their technical support on *N-myc* gene fluorescence *in-situ* hybridization analysis.

References

1. Khan AN, Solomon SS, Childress RD. Composite pheochromocytoma-ganglioneuroma: a rare experiment of nature. Endocr Pract **16**: 291-299, 2010.
2. Comstock JM, Willmore-Payne C, Holden JA, Coffin CM. Composite pheochromocytoma: a clinicopathologic and molecular comparison with ordinary pheochromocytoma and neuroblastoma. Am J Clin Pathol **132**: 69-73, 2009.
3. Lam KY, Lo CY. Composite pheochromocytoma-ganglioneuroma of the adrenal gland: an uncommon entity with distinctive clinicopathologic features. Endocr Pathol **10**: 343-352, 1999.
4. Strong VE, Kennedy T, Al-Ahmadie H, et al. Prognostic indicators of malignancy in adrenal pheochromocytomas: clinical, histopathologic, and cell cycle/apoptosis gene expression analysis. Surgery **143**: 759-768, 2008.
5. Mahajan H, Lee D, Sharma R, et al. Composite phaeochromocytoma-ganglioneuroma, an uncommon entity: report of two cases. Pathology **42**: 295-298, 2010.
6. Shawa H, Elsayes KM, Javadi S, Sircar K, Jimenez C, Habra MA. Clinical and radiologic features of pheochromocytoma/ganglioneuroma composite tumors: a case series with comparative analysis. Endocr Pract **20**: 864-869, 2014.
7. Menon S, Mahajan P, Desai SB. Composite adrenal medullary tumor: a rare cause of hypertension in a young male. Urol Ann **3**: 36-38, 2011.
8. Lau SK, Chu PG, Weiss LM. Mixed cortical adenoma and composite pheochromocytoma-ganglioneuroma: an unusual corticomedullary tumor of the adrenal gland. Ann Diagn Pathol **15**: 185-189, 2011.
9. Franquemont DW, Mills SE, Lack EE. Immunohistochemical detection of neuroblastomatous foci in composite adrenal pheochromocytoma-neuroblastoma. Am J Clin Pathol **102**: 163-170, 1994.
10. Gupta G, Saran RK, Godhi S, Srivastava S, Saluja SS, Mishra PK. Composite pheochromocytoma masquerading as solid-pseudopapillary neoplasm of pancreas. World J Clin Cases **3**: 474-478, 2015.
11. Steen O, Fernando J, Ramsay J, Prebtani APH. An unusual case of a composite pheochromocytoma with neuroblastoma. J Endocrinol Metab **4**: 39-46, 2014.
12. Royer N, Miron J, Kirby P, Weigel RJ, Lal G. Incidental finding of composite pheochromocytoma-ganglioneuroma: successful management after emergent appendectomy and review of the literature - case reports. World J Endocr Surg **3**: 39-44, 2011.
13. Kimura N, Watanabe T, Fukase M, Wakita A, Noshiro T, Kimura I. Neurofibromin and NF1 gene analysis in composite pheochromocytoma and tumors associated with von Recklinghausen's disease. Mod Pathol **15**: 183-188, 2002.
14. Rao RN, Singla N, Yadav K. Composite pheochromocytoma-ganglioneuroma of the adrenal gland: a case report with immunohistochemical study. Urol Ann **5**: 115-118, 2013.
15. Kikuchi Y, Wada R, Sakihara S, Suda T, Yagihashi S. Pheochromocytoma with histologic transformation to composite type, complicated by watery diarrhea, hypokalemia, and achlorhydria syndrome. Endocr Pract **18**: e91-e96, 2012.
16. Hu J, Wu J, Cai L, et al. Retroperitoneal composite pheochromocytoma-ganglioneuroma: a case report and review of literature. Diagn Pathol **8**: 63, 2013.
17. Chen CH, Boag AH, Beiko DT, Siemens DR, Froese A, Isotalo PA. Composite paraganglioma-ganglioneuroma of the urinary bladder: a rare neoplasm causing hemodynamic crisis at tumour

resection. Can Urol Assoc J **3**: E45-E48, 2009.

18. Gong J, Wang X, Chen X, et al. Adrenal and extra-adrenal non-functioning composite pheochromocytoma/paraganglioma with immunohistochemical ectopic hormone expression: comparison of two cases. Urol Int **85**: 368-372, 2010.

19. Hirasaki S, Kanzaki H, Okuda M, Suzuki S, Fukuhara T, Hanaoka T. Composite paraganglioma-ganglioneuroma in the retroperitoneum. World J Surg Oncol **7**: 81-81, 2009.

20. Fritzsche FR, Bode PK, Koch S, Frauenfelder T. Radiological and pathological findings of a metastatic composite paraganglioma with neuroblastoma in a man: a case report. J Med Case Rep **4**: 374, 2010.

21. Tohme CA, Mattar WE, Ghorra CS. Extra-adrenal composite pheochromocytoma-ganglioneuroma. Saudi Med J **27**: 1594-1597, 2006.

22. Yoshimi N, Tanaka T, Hara A, Bunai Y, Kato K, Mori H. Extra-adrenal pheochromocytoma-ganglioneuroma. A case report. Pathol Res Pract **188**: 1098-1100; discussion 1101-1103, 1992.

23. de Montpréville VT, Mussot S, Gharbi N, Dartevelle P, Dulmet E. Paraganglioma with ganglioneuromatous component located in the posterior mediastinum. Ann Diagn Pathol **9**: 110-114, 2005.

24. Pytel P, Krausz T, Wollmann R, Utset MF. Ganglioneuromatous paraganglioma of the cauda equina: a pathological case study. Hum Pathol **36**: 444-446, 2005.

25. Thiel EL, Trost BA, Tower RL. A composite pheochromocytoma/ganglioneuroblastoma of the adrenal gland. Pediatr Blood Cancer **54**: 1032-1034, 2010.

26. Peuchmaur M, d'Amore ES, Joshi VV, et al. Revision of the international neuroblastoma pathology classification: confirmation of favorable and unfavorable prognostic subsets in ganglioneuroblastoma, nodular. Cancer **98**: 2274-2281, 2003.

27. Nakagawara A, Ikeda K, Tsuneyoshi M, Daimaru Y, Enjoji M. Malignant pheochromocytoma with ganglioneuroblastoma elements in a patient with von Recklinghausen's disease. Cancer **55**: 2794-2798, 1985.

28. Donadio F, La Ganga V, Vajo M, Campanella G, Coverlizza S. Ganglioneuroblastoma with areas of pheochromocytoma of the adrenal gland. Case report. Minerva Chir **37**: 551-558, 1982.

29. Nigawara K, Suzuki T, Tazawa H, et al. A case of recurrent malignant pheochromocytoma complicated by watery diarrhea, hypokalemia, achlorhydria syndrome. J Clin Endocrinol Metab **65**: 1053-1056, 1987.

30. Tischler AS, Dayal Y, Balogh K, Cohen RB, Connolly JL, Tallberg K. The distribution of immunoreactive chromogranins, S-100 protein, and vasoactive intestinal peptide in compound tumors of the adrenal medulla. Hum Pathol **18**: 909-917, 1987.

31. Kakimoto S, Sakai H, Kondo A, Nishimori H, Itoh M, Kishikawa M. Malignant pheochromocytoma with ganglioneuroblastomatous elements: a case report. Nishinihon J Urol **50**: 661-665, 1988.

32. Kimura N, Miura Y, Miura K, et al. Adrenal and retroperitoneal mixed neuroendocrine-neural tumors. Endocr Pathol **2**: 139-147, 1991.

33. Watanabe T, Noshiro T, Kusakari T, et al. Two cases of pheochromocytoma diagnosed histopathologically as mixed neuroendocrine-neural tumor. Intern Med **34**: 683-687, 1995.

34. Matias-Guiu X, Garrastazu MT. Composite phaeochromocytoma-ganglioneuroblastoma in a patient with multiple endocrine neoplasia type IIA. Histopathology **32**: 281-282, 1998.

35. Fujiwara T, Kawamura M, Sasou S, Hiramori K. Results of surgery for a compound adrenal tumor consisting of pheochromocytoma and ganglioneuroblastoma in an adult: 5-year follow-up. Intern Med **39**: 58-62, 2000.

36. Okumi M, Ueda T, Ichimaru N, Fujimoto N, Itoh K. [A case of composite pheochromocytoma-ganglioneuroblastoma in the adrenal gland with primary hyperparathyroidism]. Hinyokika Kiyo **49**: 269-272, 2003 (in Japanese, Abstract in English).

37. Pathmanathan N, Murali R. Composite phaeochromocytoma with intratumoural metastatic squamous cell carcinoma. Pathology **35**: 263-265, 2003.

38. Obara Y, Nagasawa R, Nemoto W, et al. ERK5 induces ankrd1 for catecholamine biosynthesis and homeostasis in adrenal medullary cells. Cell Signal **28**: 177-189, 2016.

39. Thompson LD. Pheochromocytoma of the Adrenal gland Scaled Score (PASS) to separate benign from malignant neoplasms: a clinicopathologic and immunophenotypic study of 100 cases. Am J Surg Pathol **26**: 551-566, 2002.

40. Ichiyanagi O, Ito H, Takai S, et al. A GRIA2 and PAX8-positive renal solitary fibrous tumor with NAB2-STAT6 gene fusion. Diagn Pathol **10**: 155, 2015.

41. Wang M, Zhou C, Cai R, Li Y, Gong L. Copy number gain of MYCN gene is a recurrent genetic aberration and favorable prognostic factor in Chinese pediatric neuroblastoma patients. Diagn Pathol **8**: 5, 2013.

42. Leung K, Stamm M, Raja A, Low G. Pheochromocytoma: the range of appearances on ultrasound, CT, MRI, and functional imaging. AJR Am J Roentgenol **200**: 370-378, 2013.

43. Raja A, Leung K, Stamm M, Girgis S, Low G. Multimodality imaging findings of pheochromocytoma with associated clinical and biochemical features in 53 patients with histologically confirmed tumors. AJR Am J Roentgenol **201**: 825-833, 2013.

44. Lattin GE Jr, Sturgill ED, Tujo CA, et al. From the radiologic pathology archives: adrenal tumors and tumor-like conditions in the adult: Radiologic-pathologic correlation. Radiographics **34**: 805-829, 2014.

45. Lonergan GJ, Schwab CM, Suarez ES, Carlson CL. Neuroblastoma, ganglioneuroblastoma, and ganglioneuroma: radiologic-pathologic correlation. Radiographics **22**: 911-934, 2002.

46. Layfield LJ, Glasgow BJ, Du Puis MH, Bhuta S. Aspiration cytology and immunohistochemistry of a pheochromocytoma-ganglioneuroma of the adrenal gland. Acta Cytol **31**: 33-39, 1987.

47. Tanaka T, Yoshimi N, Iwata H, et al. Fine-needle aspiration cytology of pheochromocytoma-ganglioneuroma of the organ of Zuckerkandl. Diagn Cytopathol **5**: 64-68, 1989.

48. Ayala-Ramirez M, Feng L, Johnson MM, et al. Clinical risk factors for malignancy and overall survival in patients with pheochromocytomas and sympathetic paragangliomas: primary tumor size and primary tumor location as prognostic indicators. J Clin Endocrinol Metab **96**: 717-725, 2011.

49. Satake H, Inoue K, Kamada M, Watanabe H, Furihata M, Shuin T. Malignant composite pheochromocytoma of the adrenal gland in a patient with von Recklinghausen's disease. J Urol **165**: 1199-1200, 2001.

50. Rha SE, Byun JY, Jung SE, Chun HJ, Lee HG, Lee JM. Neurogenic tumors in the abdomen: tumor types and imaging characteristics. Radiographics **23**: 29-43, 2003.

51. Spitz R, Hero B, Simon T, Berthold F. Loss in chromosome 11q identifies tumors with increased risk for metastatic relapses in localized and 4S neuroblastoma. Clin Cancer Res **12**: 3368-3373, 2006.

52. Angelini P, London WB, Cohn SL, et al. Characteristics and outcome of patients with ganglioneuroblastoma, nodular subtype: a report from the INRG project. Eur J Cancer **48**: 1185-1191, 2012.

53. Jrebi NY, Iqbal CW, Joliat GR, Sebo TJ, Farley DR. Review of our experience with neuroblastoma and ganglioneuroblastoma in adults. World J Surg **38**: 2871-2874, 2014.

54. Bolzacchini E, Martinelli B, Pinotti G. Adult onset of ganglioneuroblastoma of the adrenal gland: case report and review of the literature. Surg Case Rep **1**: 79, 2015.

55. Look AT, Hayes FA, Shuster JJ, et al. Clinical relevance of tumor cell ploidy and N-myc gene amplification in childhood neuroblastoma: a Pediatric Oncology Group study. J Clin Oncol **9**: 581-591, 1991.

56. George RE, London WB, Cohn SL, et al. Hyperdiploidy plus nonamplified MYCN confers a favorable prognosis in children 12 to 18 months old with disseminated neuroblastoma: a Pediatric Oncology Group study. J Clin Oncol **23**: 6466-6473, 2005.

57. de Wailly P, Oragano L, Rade F, et al. Malignant pheochromocytoma: new malignancy criteria. Langenbecks Arch Surg **397**: 239-246, 2012.

58. Shuch B, Ricketts CJ, Metwalli AR, Pacak K, Linehan WM. The genetic basis of pheochromocytoma and paraganglioma: implications for management. Urology **83**: 1225-1232, 2014.

59. Armstrong R, Greenhalgh KL, Rattenberry E, et al. Succinate dehydrogenase subunit B (*SDHB*) gene deletion associated with a composite paraganglioma/neuroblastoma. J Med Genet **46**: 215-216, 2009.