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# Mixed Exocrine-Neuroendocrine Carcinoma of the Nasal Cavity: Clinico-Pathologic and Molecular Study of a Case and Review of the Literature

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**Abstract** Sinonasal intestinal-type adenocarcinomas (ITACs) are rare neoplasms histologically resembling intestinal adenocarcinomas. Although a neuroendocrine differentiation in ITACs has been described, true mixed exocrine-neuroendocrine carcinomas, neoplasms in which each component represents at least 30 % of the lesion, are extremely rare and their molecular alterations are largely unknown. We describe herein the clinico-pathologic features, the methylation profile, chromosomal gains and losses, and mutation analysis of KRAS, BRAF and p53 in a nasal mixed exocrine-neuroendocrine carcinoma resected in a 79-year-old man. The tumor was composed of an ITAC and a poorly differentiated neuroendocrine carcinoma. Both exocrine and neuroendocrine components were CK8, CK20, CDX2 and p53 positive, and CK7 and TTF1 negative. The neuroendocrine component also showed immunoreactivity for chromogranin A, synaptophysin, serotonin and glicentin. Gains and losses were found at following chromosome regions: 17p13 (*TP53*), 14q24 (*MLH3*), 19q13 (*KLK3*), 5q21 (*APC*), 7q21 (*CDK6*),

9q34 (*DAPK1*), 12p13 (*TNFRSF 1A*, *CDKN1B*), 13q12 (*BRCA2*), 17p13.3 (*HIC1*), 18q21 (*BCL2*), and 22q12 (*TIMP3*). Aberrant methylation was detected only in the neuroendocrine component and involved *APC* and *DAPK1* genes. No mutation of KRAS (exons 2–4), BRAF (exon 15), and p53 (exons 4–10) was found in both components. The results suggest a monoclonal origin of the tumor from a pluripotent cell undergoing a biphenotypic differentiation and that the neuroendocrine differentiation may be from an exocrine to an endocrine pathway. We have also reviewed the literature on sinonasal mixed exocrine-neuroendocrine carcinomas to give to the reader a comprehensive overview of these very rare tumor types.

**Keywords** Intestinal-type adenocarcinomas · Neuroendocrine carcinoma · Nasal cavity · Mixed adenoneuroendocrine carcinoma · Molecular profile

## Introduction

Sinonasal epithelial malignant tumors are rare neoplasms accounting for less than 1:100,000 population per year and represent about 0.2–0.8 % of all malignant neoplasms [1]. Among various different histological tumor types that can originate in the sinonasal region, intestinal-type adenocarcinoma (ITAC) is a peculiar tumor which histologically resembles intestinal adenocarcinoma and is associated with specific occupational exposure including wood and leather dusts. Although the frequency among primary sinonasal malignancies and the true incidence are difficult to ascertain due to the rarity of such cancer, a male predominance and a mean patient age of 58 years have been established [2].

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As in intestinal adenocarcinomas, the recognition of a neuroendocrine differentiation has been also described in nasal ITACs [3–6]. In gut adenocarcinomas a wide spectrum of combinations of both neuroendocrine and exocrine components has been accurately described [7], but mixed exocrine-neuroendocrine carcinomas, now classified as mixed adenoneuroendocrine carcinomas (MANECs), are by definition neoplasms in which each component represents at least 30 % of the lesion [8]. Only seven nasal MANECs have been described in the literature to date [9–13], but this entity was not included in the WHO classification of tumors of the head and neck, probably due to its rarity [1]. However, considering the close analogies between intestinal and sinonasal adenocarcinomas and considering that in the gut MANEC is regarded as a specific entity [8] this may also represent a distinct clinico-pathologic type in the sinonasal cavity.

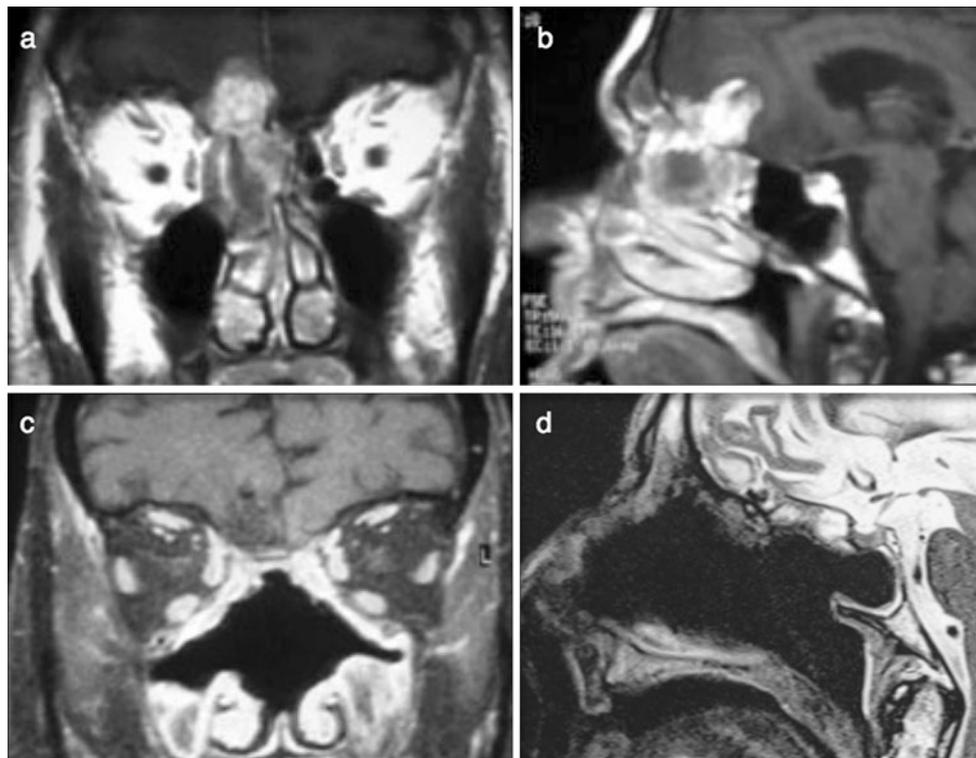
So far, only a few studies addressed the molecular alterations in ITACs and no data are available about the pathogenesis of this tumor type. Despite its histological similarity to colorectal carcinomas, the genetic and epigenetic changes frequently involved in colorectal tumorigenesis have been demonstrated at lower frequency in ITACs [14–17]. Other investigations reported a close relationship between dust exposure and specific gene

alterations, including promoter methylation of p14 and p16 as well as *TP53* mutation and/or loss of 17p13 region [18].

In this report, we describe the clinico-pathologic, genetic and epigenetic features of a sinonasal ITAC with an abundant neuroendocrine component and we review the literature of this subject with the aim of better characterizing this rare tumor type.

### Case Report

A 79-year-old patient underwent head and neck computer tomography (CT) scan because of a transitory ischemic attack and left arm weakness. The scan showed a right ethmoidal mass with intracranial involvement. The patient was a paperhanger and did not report respiratory airway obstruction, rhinorrhea, epistaxis or other nasal symptoms. To define the local extension of the disease more precisely, the patient also underwent head magnetic resonance imaging which showed an irregular hourglass shape lesion involving the ethmoidal complex and the right supraorbital recess, with a large intracranial extension apparently compressing the frontal lobe without infiltration of the brain parenchyma (Fig. 1). Endoscopic examination revealed a grayish ulcerated mass filling the right ostio-meatal



**Fig. 1** The pre-operative magnetic resonance scan shows an irregular hourglass shape lesion involving the ethmoidal complex with a large intradural and intracranial extension (a, b). The post-operative

magnetic resonance scan demonstrates the radical resection of the sinonasal and intracranial lesion (c, d)

complex, a biopsy was performed and the histological examination revealed an ITAC with a diffuse neuroendocrine component (see Results section for details). Staging of the disease included neck ultrasound, bone scintigraphy and total-body CT which did not indicate metastatic disease. The patient was considered eligible for a mini-invasive surgical treatment and underwent endoscopic endonasal tumor resection with trans-nasal craniectomy (Fig. 1). The disease was staged as pT4bNOM0 and postoperative radiotherapy was planned in a 54 Gy dose on the primary site of the tumor. No chemotherapy regimen was proposed because of the comorbidity of the patient. The patient was free of disease until the 26th month after surgery when he developed bone metastases and died soon after. Autopsy was not performed.

**Materials and Methods**

**Morphology and Immunohistochemistry**

Tumor tissue was fixed in buffered formalin (formaldehyde 4 % w/v and acetate buffer 0.05 M) and routinely processed to paraffin wax. Serial sections were stained with hematoxylin and eosin (H&E) and Alcian-blue/periodic acid Schiff (AB-PAS) stains for the histopathologic evaluation. For immunohistochemistry, 5 µm-thick sections were mounted on poly-L-lysine coated slides, deparaffinized, quenched with 3 % hydrogen peroxide for 10 min and then incubated with primary antibodies (Table 1) at

4 °C for 18–20 h, followed by the avidin–biotin complex (ABC) procedure. Immunoreactions were developed using 0.03 % 3,3'-diaminobenzidine tetrahydrochloride and then sections were counterstained with Harris' hematoxylin.

**Methylation –Specific Multiplex Ligation Probe Amplification (MS-MLPA)**

Microspecimens of the exocrine and the endocrine component were manually microdissected from three 8 µm-cut histologic sections in order to obtain at least 70 % of tumor cells in both samples. DNA was extracted using a QIAamp® DNA FFPE Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Tumor DNA was obtained from formalin fixed and paraffin embedded tissue. The MS-MLPA assay was used to assess in both the tumor components the methylation status of 34 genes and copy number changes of 51 different DNA sequences (Table 2). The MS-MLPA analysis was performed using the two commercial kits SALSA MS-MLPA ME001 Tumor suppressor-1 Kit and SALSA MS-MLPA ME002 Tumor suppressor-2 Kit (MRC-Holland, Amsterdam, The Netherlands) according to the protocol previously reported [19]. Copy number and methylation variation were detected using Coffalyser V7 software (MRC-Holland). Identification of copy number changes was determined according to recommendations from MRC-Holland. Methylation dosage ratio (MR) was obtained by the following calculation:  $MR = (P_x/P_{ctrl})_{Dig}/(P_x/P_{ctrl})_{Undig}$  where  $P_x$  is the peak area of a given target probe,  $P_{ctrl}$  is the sum of the peak areas of all control

**Table 1** Antibodies and antisera used

Antibodies/antisera	P/M(Clone)	Dilution	Source
Synaptophysin	M (snp88)	1:100	BioGenex Laboratories, San Ramon, CA, USA
Chromogranin A	M (LK2H10)	1:100	Ventana, Tucson, AZ, USA
Cytokeratin	M (AE1/AE3)	1:1	Ventana, Tucson, AZ, USA
Cytokeratin 7	M (OV-TL12/30)	1:200	Dako, Glostrup, Denmark
Cytokeratin 8/18	M (35BH11)	1:100	Dako
Cytokeratin 20	M (Ks 20.8)	1:100	Dako
Glicentin	P	1:7500	Milab, Malmoe, Sweden
Somatostatin	P	1:1500	Dako
Pancreatic polypeptide	P	1:4000	Cambridge Research Biochemicals, Cambridge, UK
Serotonin	M (YC5)	1:50	Biogenesis, Bournemouth, UK
TTF1	M (SPT24)	1:100	Novocastra, New Castle, UK
CDX2	M (CDX2-88)	1:100	BioGenex Laboratories
CEA	M (TF3H8-1)	1:1	Ventana, Tucson, AZ, USA
APC	P	1:800	Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA
P53	M (D07)	1:500	Dako
Ki67	M(MIB1)	1:50	Dako

P/M polyclonal/monoclonal

**Table 2** Copy number variations and promoter methylation analysis in the ITAC and NEC components

Chromosome region	Gene	ITAC component			NEC component		
		met	loss	gain	met	loss	gain
01p36	TP73						
02q33-q34	CASP8						
03p14.2	FHIT						
03p21.3	MLH1						
03p21.3	RASSF1						
03p22	CTNNB1	na			na		
03p24	RARB						
03p26-p25	VHL						
03q21	CASR	na			na		
04q26	IL2	na			na		
05q21	APC						
06q25.1	ESR1						
06q26	PARK2	na			na		
07q21.3	CDK6	na			na		
09p13	PAX5A						
09p21	CDKN2A (p14)						
09p21	CDKN2A (p16)						
09p21	CDKN2B						
09q22.3	PTCH1	na			na		
09q34.1	DAPK1						
10p12.1	CREM	na			na		
10q23.31	PTEN						
10q26	MGMT						
11p12	CD44						
11p13	PAX6						
11p13	WT1						
11p13	CD44						
11q13	GSTP1						
11q22.3	ATM						
11q23	IGSF4						
12p13	TNFRSF 1A	na			na		
12p13.1	CDKN1B						
12q23	PAH	na			na		
12q24.33	CHFR						
13q12.3	BRCA2						
13q14.2	RB1						
14q24.3	MLH3	na			na		
15q15	THBS1						
16p12	PYCARD						
16p13.3	TSC2	na			na		
16q22.1	CDH1	na			na		
16q24.2	CDH13						
17p12	PMP 22	na			na		
17p13.1	TP53						
17p13.3	HIC1						
17q21	BRCA1						
18q21.3	BCL2	na			na		
19p13.3	STK11						
19q13	KLK3	na			na		
20q13.33	GATA5						
22q12.3	TIMP3						

Met: methylation analysis; na: finding not available for the methylation status because the 15 reference probes do not contain *HhaI* digestion sites; the black boxes indicate the presence of methylation or of copy number variation depending on the specific column; the white boxes indicate the absence of methylation or of copy number variation

probes, Dig stands for HhaI digested sample, and Undig stands for undigested sample. Based on our previous validation experiments aberrant methylation was scored as a categorical variable using a specific MR threshold for each gene corresponding to the highest level of accuracy of the test [19].

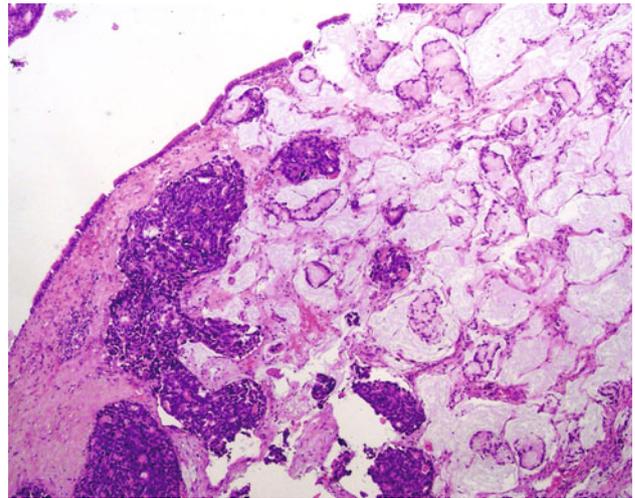
#### Mutational Analysis of KRAS, BRAF and TP53 Genes

KRAS mutations at codons 12, 13, 61 and 146 and BRAF at codon 600 were analyzed by pyrosequencing using an Anti EGFR MoAb response<sup>®</sup> (KRAS status) kit and an Anti EGFR MoAb response<sup>®</sup> (BRAF status) kit (Diatech Pharmacogenomics, Jesi, Italy). Exons 2–4 of KRAS and exon 15 of BRAF were PCR amplified according to the manufacturer's instructions. Real-time reactions and post-PCR melting curve analysis were performed using a Rotor Gene 6000 (Corbett Research, Sydney, Australia). Pyrosequencing was carried out with Pyrogold reagents on a PyroMark Q96 ID system (Qiagen) according to the manufacturer's protocol. Pyrogram outputs were analyzed by the Pyromark Q24 software (Qiagen, Hilden, Germany) using the Allele Quantification software to determine the percentage of mutant versus wild-type alleles according to percentage relative peak height.

The specimens were also screened for the presence of TP53 mutations in exons 4–10 by direct sequencing as previously described [20]. Sequences were confirmed at least twice starting from independent PCR reactions, by comparing sense and antisense strands.

#### Results

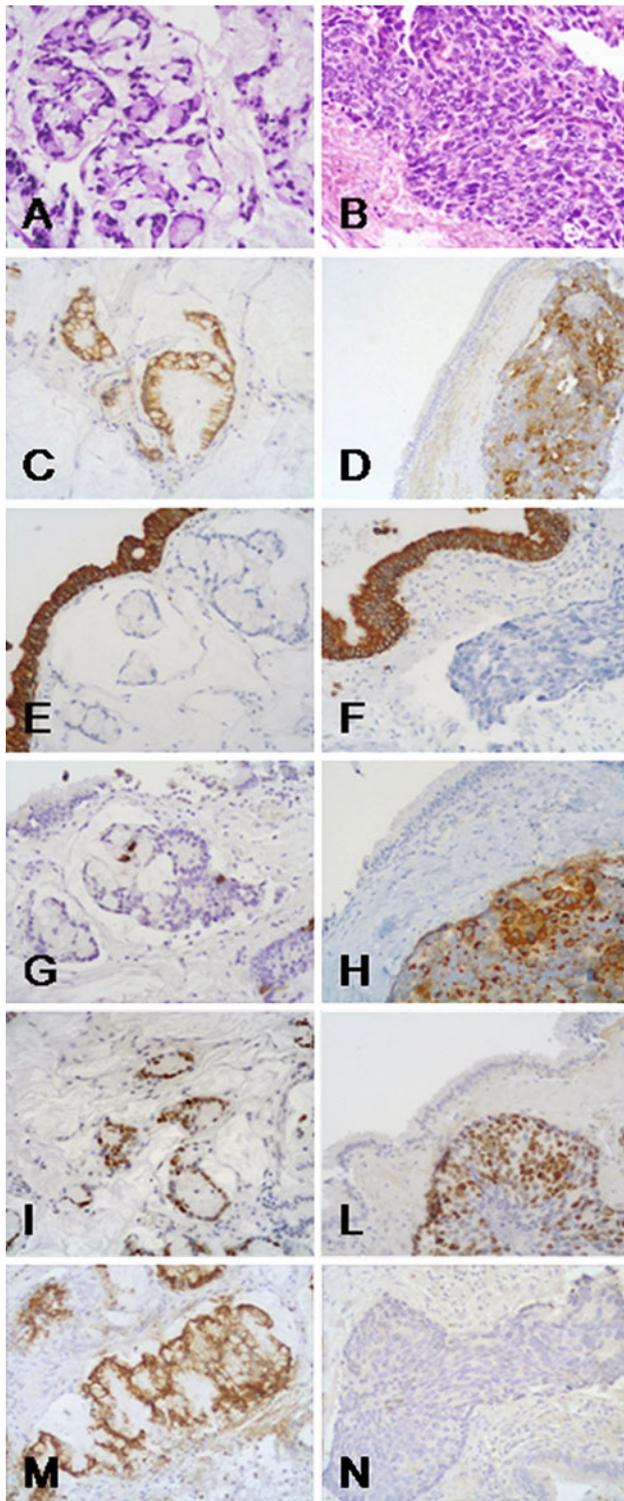
The tumor was formed of two components which were very close to each other or even fused in some areas (Fig. 2). One component was a moderately differentiated ITAC with an abundant mucinous component; it was characterized by a proliferation of invasive glandular structures lined by stratified columnar cells with variably pleomorphic and hyperchromatic nuclei closely resembling colorectal adenocarcinoma (Fig. 3). Areas of mucinous lakes with glands inside were observed. The neuroendocrine component (Fig. 3), showing the typical features of a poorly differentiated neuroendocrine carcinoma [8, 21], was characterized by a proliferation of poorly differentiated small to intermediate cells forming solid nests. Cells showed hyperchromic and atypical nuclei with a high nuclear/cytoplasmic ratio, inconspicuous to absent nucleoli, and a high mitotic count (23 mitoses X 10HPF). The neuroendocrine component showed a diffuse immunoreactivity for synaptophysin and chromogranin A, which was lacking in the adenocarcinoma



**Fig. 2** Panoramic view of the tumor that was formed of two components which were very close to each other. The exocrine component (*right*) was a moderately differentiated ITAC with an abundant mucinous component with areas of mucinous lakes with glands inside. The neuroendocrine component (*left*) was characterized by solid sheets of poorly differentiated neuroendocrine cells

with the exception of a few scattered cells in some glandular structures identified with both antibodies. In addition, neuroendocrine tumor cells presented immunoreactivity for serotonin and glicentin, whereas they were negative for pancreatic polypeptide. Cytokeratin (CK) 8, CK20, CDX2 were expressed in both neoplastic components, which were conversely negative for CK7 and TTF1 (Fig. 3). Since each component represented more than 30 % of the tumor tissue the neoplasm was classified as a mixed exocrine-neuroendocrine carcinoma [22] or, following the recent proposal of the 2010 WHO classification [8], as a mixed adenoneuroendocrine carcinoma (MANEC). Peritumoral sino-nasal mucosa did not show any dysplastic lesion.

The MS-MLPA analysis was used to investigate the genetic and epigenetic profiles of the two tumor components. MS-MLPA results are summarized in Table 2. Concurrent copy number changes in the endocrine and exocrine components were observed at 17p13 (*TP53*), 14q24 (*MLH3*) and 19q13 (*KLK3*) regions. In agreement with the molecular result, an immunohistochemical p53 accumulation was observed in both neoplastic components (Fig. 3). Additional gains and losses were found to be restricted to the neuroendocrine component and included copy number variations at 5q21 (*APC*), 7q21 (*CDK6*), 9q34 (*DAPK1*), 12p13 (*TNFRSF 1A*, *CDKN1B*), 13q12 (*BRCA2*), 17p13.3 (*HIC1*), 18q21 (*BCL2*), 19q13 (*KLK3*) and 22q12 (*TIMP3*) regions. Aberrant methylation was detected only in the neuroendocrine component and involved *APC* and *DAPK1* genes. Interestingly, the biallelic inactivation of the *APC* gene (by deletion and aberrant



**Fig. 3** The exocrine component is a moderately differentiated ITAC characterized by a proliferation of invasive glandular structures lined by stratified columnar cells with variably pleomorphic and hyperchromatic nuclei, closely resembling colorectal adenocarcinoma (a). The neuroendocrine component, showing the typical features of a NEC, is characterized by a proliferation of poorly differentiated small to intermediate cells forming solid nests (b). Both components were immunoreactive for cytokeratin 20 (c, d), but not for cytokeratin 7 (e, f; the normal nasal epithelium serves as positive internal control). In the ITAC component only scattered chromogranin A positive cells were observed (g), while the NEC component showed an intense chromogranin A immunoreactivity (h). Both components were p53 positive (i, l). APC immunoreactivity was observed in the ITAC component (m), while it was lacking in the NEC component (n)

mutational analysis of KRAS (codon 12, 13, 61 and 146), BRAF (codon 600) and TP53 (exons 4–10) did not find any mutation in each component of the tumor.

### Discussion

In the last 40 years, the systematic application of immunohistochemical techniques to the study of tumors has led to the recognition that neuroendocrine cells occur rather frequently in exocrine neoplasms [23–25] and, especially, in adenocarcinomas of the digestive system [7, 26, 27]. It is now well known that there is a wide spectrum of combination of exocrine and neuroendocrine components, ranging from adenomas or carcinomas with interspersed neuroendocrine cells at one extreme to classical neuroendocrine tumors with focal exocrine component at the other [7, 26]. In addition, both exocrine and neuroendocrine components can show different morphological features ranging, for the former, from adenoma to adenocarcinoma with different degrees of differentiation and, for the latter, from well differentiated to poorly differentiated neuroendocrine tumors [7]. Although this spectrum of combinations of neuroendocrine and exocrine components is frequently observed in routine practice, mixed exocrine-neuroendocrine tumors are rare and are, by definition, neoplasms in which each component represents at least 30 % of the lesion [8, 22]. The clinical significance and the influence on survival of focal neuroendocrine differentiation in gut adenocarcinomas still remain controversial. Conversely, gastrointestinal mixed exocrine-neuroendocrine tumors can be stratified in different prognostic categories according to the grade of malignancy of each component [7]. Antibodies directed against chromogranin A and synaptophysin are the most frequently used to identify the neuroendocrine component.

Although the recognition of a neuroendocrine differentiation in nasal ITACs has been described [3–6], mixed adenoneuroendocrine carcinomas (MANECs) have been poorly reported accounting for only seven cases described

methylation, respectively) was observed only in the neuroendocrine component (Table 2). This result was confirmed by immunohistochemistry showing a diffuse immunoreactivity for APC protein in the ITAC component, which was lacking in the NEC component (Fig. 3). The

**Table 3** Literature review: sinonasal mixed adenoneuroendocrine carcinomas

	Histologic components		Sex	Age	Symptoms	Occupational exposure	Metastases at diagnosis	Therapy	Follow-up (months)	Reference
	Endocrine	Exocrine								
1	NEC	ADC	nk	nk	nk	No	Lung, kidney, adrenal, liver, node	nk	nk	[12]
2	NET	ITAC	M	52	Headache	No	No*	S + RT	DOD (30)	[10]
3	NEC	ADC	nk	nk	nk	nk	nk	nk	nk	[13]
4	NEC	ADC	M	51	nk	nk	No	C + RT	AWD (12)	[9]
5	NEC	Inv Pap	M	57	nk	nk	No	S	DOD (6)	[9]
6	NEC	ITAC	M	50	Stuffiness, rhinorrhea	No	Node	S + C + RT	nk	[11]
7	NEC	ITAC	M	75	Epistaxis, rhinorrhea,	No	No	S	DOD (4)	[11]
8	NEC	ITAC	M	79	TIA	No	No*	S + RT	DOD (26)	Present case

ADC adenocarcinoma, ITAC intestinal-type adenocarcinoma, Inv Pap inverted papilloma, C chemotherapy, RT radiotherapy, S surgery, nk not known, M male, \* infiltration of anterior cranial fossa, NEC poorly differentiated neuroendocrine carcinoma, NET neuroendocrine tumor, TIA transitory ischemic attack, DOD died of disease, AWD alive with disease

in the English literature [9–13]. Moreover, this entity was not included in the recent WHO classification of tumors of the head and neck, probably because of its rarity [1]. However, considering the close analogies between intestinal and sinonasal intestinal-type adenocarcinomas and considering that in the gut MANEC is considered as a specific entity [8], it is reasonable to suggest that this is also a distinct clinico-pathologic type in the sinonasal cavity. From the review of the literature (Table 3) it appears that sinonasal mixed exocrine-neuroendocrine tumors can show different types of components. With the exception of one case [10] the neuroendocrine component was a poorly differentiated neuroendocrine carcinoma (NEC). The exocrine component was most often an ITAC, even though three not otherwise specified adenocarcinomas [9, 12, 13] and one inverted papilloma [9] were also reported. MANECs were found in males at a mean age of 60.6 years (range 51–79 years). Based on the limited available information, it would seem that sinonasal MANECs are not associated with occupational risks in the same way as sinonasal ITACs. Two tumors showed lymph node or distant metastases at the time of diagnosis, while other two cancers presented infiltration of the anterior cranial fossa. Two neoplasms developed bone metastases during follow-up. Survival information was available for five patients, all but one died of disease after a mean time of 16.5 months (range 4–30 months). From this analysis it appears that sinonasal MANECs can be considered aggressive neoplasms associated with poor prognosis. In this respect, a clear definition of a molecular profile may have a clinical influence in terms of tailored complementary therapy to associate with surgical procedure.

The immunophenotype of the exocrine component of the present tumor, including CK20, CK8, p53 and CDX2 immunoreactivity and the lack of TTF1 and CK7 expression, are in line with previous findings [3, 28], although CK7 expression has also been reported in some ITACs [11, 29]. The immunohistochemical profile of the neuroendocrine component largely overlapped with that of the exocrine one, but also showed a typical neuroendocrine phenotype including diffuse and intense immunoreactivity for chromogranin A and synaptophysin. These two neuroendocrine markers are generally widely applied to define the neuroendocrine nature of a tumor. Chromogranin A is present in the majority on neuroendocrine neoplasms even if tumors containing only a small number of secretory granules (i.e. NECs) may only exhibit a weak immunoreactivity for this marker. For this reason, synaptophysin expression should be included in the diagnostic panel of poorly differentiated carcinomas, because its immunoreactivity is independent of the presence of secretory granules and shows a higher sensitivity than chromogranin A expression [30]. Interestingly, the expression of glicentin, a hormonal peptide mainly expressed by neuroendocrine tumors of hindgut derivation [31], suggests a similarity between the neuroendocrine component and gut neuroendocrine neoplasms arising in the distal bowel. The CDX2 expression observed in the NEC component underlines the intestinal phenotype of the neuroendocrine component [19].

In agreement with these results, the molecular characterization of the tumor by the MS-MLPA assay strongly supported the involvement of the APC pathway in the progression of the cancer, confirming a genetic resemblance with colorectal carcinomas. Our data are consistent

with previous studies demonstrating that ITACs share genetic similarities with microsatellite stable colorectal cancers [32, 33]. Until now, the involvement of APC in ITACs has been poorly examined. To our knowledge, a single mutational study of the gene has been performed, reporting only missense mutations, which might not affect the APC protein activity [32]. In this study, we demonstrated a concurrent hypermethylation and allelic loss of APC that determined lack of protein expression in the neuroendocrine component. By contrast, no gene or protein alterations were observed in the ITAC component (Fig. 2). This result suggests a causal role of APC in the progression of these tumors and outlines the potential utility of further analyses of APC promoter hypermethylation or loss of heterozygosity in ITACs and in MANECs. Promoter hypermethylation of DAPK1 was also found to be restricted to the neuroendocrine component. Interestingly, the aberrant methylation of DAPK1 is considered an important epigenetic event in nasopharyngeal carcinomas [34] and has been evaluated as an early molecular marker of sub-clinical presence of this neoplasm [35, 36].

Regarding the *KRAS/BRAF* pathway, our analysis did not find any mutation in the hotspot codons of the two genes. This result was not unexpected because, in several studies, a lower frequency of *KRAS* mutations has been reported in ITACs compared with colorectal carcinomas [17]. There is less information about *BRAF* mutation, which was not found in any of the 18 cases investigated in a recent published series [32].

In this study, a strong genetic relationship between the ITAC and NEC component was observed. Indeed, both components exhibited three concurrent copy number changes at 17p13 (TP53), 14q24 (MLH3) and 19q13 (KLK3) regions, while all the additional alterations of gene dosage were restricted to the NEC. These results strongly support the hypothesis of a monoclonal origin of the tumor from a pluripotent cell that undergoes a biphenotypic differentiation after carcinogenesis is initiated, as already proposed for MANECs of the lung [37] and other sites [38, 39]. In agreement with these previous molecular studies, the NEC component showed a more complex genetic profile than the adenocarcinoma, confirming that the neuroendocrine differentiation may be from an exocrine to an endocrine cell type and not vice versa.

In conclusion, extensive genetic and epigenetic analyses of heterogeneous tumors exhibiting early and more aggressive components are very informative approaches to define the clonality and timing of the molecular events underlying tumor progression. In these applications, MS-MLPA appears to be a powerful tool to detect copy number variation and hypermethylation of a large set of genes simultaneously using small amounts of DNA, also deriving from archival tissues.

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