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ScienceDirect

Journal of the Chinese Medical Association xx (2015) 1−6

www.jcma-online.com

Original Article

High neuroendocrine component is a factor for poor prognosis in gastrointestinal high-grade malignant mixed adenoneuroendocrine neoplasms

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Received August 7, 2014; accepted January 30, 2015

Abstract

Background: High-grade malignant mixed adenoneuroendocrine carcinoma (MANEC) is a highly malignant combined neoplasm formed by an adenocarcinomatous component and a poorly differentiated (Grade 3) neuroendocrine (NE) carcinoma.
Methods: Tumors from 21 patients with gastrointestinal high-grade malignant MANECs or tumors with varying percentages of Grade 3 NE component were examined, and the NE component was confirmed by morphological analysis and immunohistochemical staining. Patients were divided into high NE (NE component $>$ 50% in the primary tumor) and low NE (NE component $\leq$ 50% in the primary tumor) component groups.
Results: High NE component was a poor prognostic factor for patients with high grade MANEC ($p = 0.021$). Out of 13 patients with high-grade malignant MANEC, eight had a pure NE component, one had a pure adenocarcinomatous component, and four had mixed-type cancer in the metastatic lymph nodes. We further enrolled eight patients who had a Grade 3 NE component in the primary tumor and found that the pure NE component in tumor emboli and distant liver metastases were more frequent in the high NE than in the low NE component group ($p = 0.012$ and $p = 0.046$, respectively).
Conclusion: The predominant tumor component in primary tumors was a prognostic factor and could predict tumor emboli and liver metastases pathology in high-grade malignant MANECs.

Keywords: high-grade malignant mixed adenoneuroendocrine carcinoma; neuroendocrine component; prognosis

1. Introduction

Human cancers with a combination of neuroendocrine (NE) and exocrine features are known to occur in various organs such as the prostate,[1] breast,[2,3] colon,[4] stomach,[5−8] and lungs.[9] This spectrum of tumors shows mixed divergent differentiation along NE and exocrine lineages; these two components exhibit variable proportion, ranging from 1% to 99%. From these

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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http://dx.doi.org/10.1016/j.jcma.2015.04.002

tumors, mixed adenoneuroendocrine carcinomas (MANECs) are defined as malignant tumors characterized by morphologically recognizable gland-forming epithelial and NE components, with at least 30% of either component being present in the tumor, according to the World Health Organization (WHO) classification of endocrine tumors of the digestive system.[10]

MANECs are very rare cancers,[11,12] and can be divided into low, intermediate, and high grades of malignancy. The optimal strategy for managing MANEC is unknown, due to the rarity of these neoplasms. In general, MANECs containing a low-grade NE tumor component and an adenocarcinomatous component should be treated as adenocarcinoma.[13−15] Tumors containing a high-grade NE component should be treated as NEC.[13−15]

It is interesting that the extent of these two components in primary tumors and lymph nodes is not always the same.[5,6] One report presented a case of gastric MANEC, in which the neoplastic signet-ring cell exocrine and NE components occurred in fairly equivalent amounts, with only the NE carcinoma portions of the tumor detected in the metastatic lymph nodes.[5] Distant metastasis to vital organs is the main cause of death from cancer. Thus, it is worth investigating the tumor components in the lymph nodes and distant metastases of patients with MANEC, which may help clinicians to adopt different treatment strategies.

In this study, we retrospectively enrolled 13 patients with high-grade malignant MANEC and observed the pathology of the primary tumor, tumor emboli, lymph nodes, and distant liver metastases. Eight patients had a pure NE component, one patient had an adenocarcinomatous component, and four patients had a mixed-type cancer in the metastatic lymph nodes. We further enrolled another eight patients who had a Grade 3 NE component in the primary tumor. Of the eight patients, the proportion of the NE component was > 70% in five patients, and < 30% in three patients. We identified that the high NE component in the primary tumor was a factor for poor prognosis and also could predict the pathology of tumor emboli and distant liver metastases in high-grade MANECs.

2. Methods

2.1. Patient clinicopathological data

This study was approved by the Ethics Committee of the Taipei Veterans General Hospital, Taipei, Taiwan and the National Taiwan University Hospital, Taipei, Taiwan. Clinical data and pathological data were obtained through a detailed retrospective review of the medical records for 13 patients with MANEC between 2000 and 2010 at the Taipei Veterans General Hospital and the National Taiwan University Hospital. All patients underwent surgery. The median age of the patients was 76 years (range, 55−84 years). The origin of the primary tumor differed between patients; the primary tumor site was the stomach in nine patients, the colon in three, and the gall bladder in one. Follow-up data were available in all cases, and the duration of follow-up was 0.8−49.7 months (median, 15.9 months; mean, 18.6 months). The last survival data were collected on December 31, 2013. We also enrolled another eight patients whose tumors had Grade 3 NE components. The percentage of the NE component was > 70% in five patients and < 30% in three patients. The characteristics of 21 cases are summarized in Table 1.

2.2. Immunohistochemical staining

Hematoxylin and eosin staining results of all 21 samples were reviewed by two pathologists. The specimens were fixed in formalin and embedded in paraffin before they were archived. We used the archived specimens for immunohistochemical analysis. Immunohistochemical staining was performed with Bond-Max autostainer (Leica Microsystems, Wetzlar, Germany), using CD56 (NCL-CD56-1B6, clone 1B6; 1:15; Novocastra/Leica, Newcastle-upon-Tyne, UK), chromogranin (NCL-CHROM-430, clone 5H7; 1:50; Novocastra/Leica), synaptophysin (NCL-SYNAP-299, clone 27G12; 1:50; Novocastra/Leica), and neuron-specific enolase (BBS/NC/VI-H14; 1:200; Dako, Carpinteria, CA, USA). Briefly, specimens from the paraffin-embedded blocks were cut into 5-μm sections. The sections were dewaxed in a 60°C oven, deparaffinized in xylene, rehydrated through serial dilutions of alcohol, and washed in phosphate-buffered saline (pH 7.2). Immunohistochemical staining was performed in the fully automated Bond-Max autostainer using onboard, heat-induced antigen retrieval in citrate buffer according to the ER1 protocol for 20

Table 1
Univariate analysis of factors influencing the overall survival (OS) of 21 high-grade mixed adenoneuroendocrine tumor patients.[a]

Characteristics	No. of patients (n = 21)	Median OS (mo)	p
Age, (y)			0.819
≥ 65	14 (66.7)	15.4	
< 65	7 (33.3)	24.9	
Sex			0.396
Male	14 (66.7)	24.7	
Female	7 (33.3)	12.0	
Tumor origin			0.711
Stomach	16 (76.2)	24.7	
Gall bladder	2 (9.5)	5.0	
Colon	3 (14.3)	10.7	
Adenocarcinoma grading			0.218
G1	5 (23.8)	15.4	
G2	14 (66.7)	24.9	
G3	2 (9.5)	1.4	
Neuroendocrine component (%)			**0.021**
> 50	13 (61.9)	10.7	
≤ 50	8 (38.1)	Not reached	
Chemotherapy			0.635
Yes	10 (47.6)	24.7	
No	11 (52.4)	15.4	
AJCC staging			0.455
I + II	5 (23.8)	Not reached	
III + IV	16 (76.2)	12.0	

Bold value signifies $p < 0.05$.
Data are presented as n (%).
AJCC = American Joint Committee on Cancer.
[a] World Health Organization classification [9].

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minutes and a VBS Refine polymer detection system (Leica Microsystems). Diaminobenzidine was used as the chromogen (Leica Microsystems). The sections were then counterstained with hematoxylin.

2.3. Evaluation of the NE component in primary tumors, tumor emboli, lymph nodes, and distant liver metastases

The percentage of the NE component in primary tumors, status of tumor spreading, and the tumor component in tumor emboli, lymph nodes or liver metastases were evaluated by two pathologists. In primary tumors, the NE component was confirmed by morphology and positive immunohistochemical staining for two of the four NE markers (CD56, chromogranin, synaptophysin, and neuron-specific enolase).

For tumors found in tumor emboli, lymph nodes, and liver metastases, the NE component was confirmed by morphology alone, the results of which were then compared with that of primary tumors. Cases of adenocarcinoma showing focal NE differentiation were identified only by immunostaining and not by morphological differences; amphicrine tumors thus identified were excluded from the study.

2.4. Statistical analysis

We divided the patients into two groups: the high NE-component group ($n = 13$; defined by an NE component of $> 50\%$ in the primary tumor) and the low NE-component group ($n = 8$; defined by an NE component of $\leq 50\%$ in the primary tumor). The association between the proportion of NE component in primary tumors and in tumor emboli or lymph node or distant liver metastases was analyzed for statistical significance using the Chi-square test. Survival data were analyzed according to the Kaplan–Meier method. The log-rank test was used to compare survival data between groups. A p value of < 0.05 was considered significant.

3. Results

3.1. High NE components in primary MANEC was associated with poor prognosis

Univariate overall survival analysis was performed to test the prognostic significance of clinical variables in 21 MANEC cases, and only NE components in primary tumor was shown to have an adverse impact on survival ($p = 0.021$; Table 1 and Fig. 1). Our data indicate that high NE components in primary tumor predicted a poor prognosis in terms of high-grade MANEC.

3.2. Pathology of tumors involved in tumor emboli was similar to that in metastatic lymph nodes and distant liver metastases

Of the 21 patients, 20 had tumor emboli, 17 had metastatic lymph nodes, and four had distant liver metastases. Pure NE sections in tumor emboli were observed in 13 patients, pure adenocarcinoma was observed in four patients, and both components were observed in three patients (Table 2). In most cases, the components of lymph node metastases and distant liver metastases were similar to those of tumor emboli; only two patients had different components (Cases 2 and 9, Table 2).

Therefore, the tumor components involved in tumor emboli may suggest the pathology of metastatic lymph nodes and distant liver metastases (Table 2).

3.3. Increased percentage of pure NE component tumors in tumor emboli and distant liver metastases in the high NE component group

Next, we divided the patients into two groups: the high NE component group with patients showing $> 50\%$ NE component in the primary tumor ($n = 13$) and the low NE component group with patients showing $\leq 50\%$ NE component in the primary tumor ($n = 8$; Table 3). In cases of tumor emboli, 91.7% (11/12) of patients in the high NE group had a pure NE component in their tumors, whereas 50% (4/8) of patients in the low NE group had a pure adenocarcinomatous component. In cases of distant liver metastases, 100% (3/3) of patients in the high NE group had a pure NE component in their tumors, whereas 100% (1/1) of patients in the low NE group had a pure adenocarcinomatous component. From these results, we observed that tumor emboli and distant liver metastases with a pure NE component were observed more frequently in the high NE component group than in the low NE component group ($p = 0.012$ and $p = 0.046$; Table 3).

3.4. Increased percentage of pure NE-component tumors in lymph nodes in high NE component group

In cases of lymph nodes, 81.8% (9/11) of patients in the high NE component group showed pure NE-component tumors in their lesions of lymph nodes, whereas 33.3% (2/6) of patients in the low NE component group showed pure adenocarcinomatous component tumors. These data show that pure NE-component tumors in lymph nodes showed more of a trend in the high NE component group than in the low NE component group ($p = 0.065$; Table 3).

4. Discussion

Gastrointestinal MANECs can be stratified into three different prognostic categories according to the grade of each component's malignancy.[16] High-grade malignant MANEC is a highly malignant combined neoplasm formed by an adenomatous (villous or tubulovillous) or carcinomatous (adenocarcinoma or squamous cell carcinoma) component, together with a poorly differentiated (Grade 3) NEC.[15] The optimal treatment strategy for high-grade malignant MANEC is unknown because of the rarity of the disease. When considering treatment, the more aggressive component of MANECs should be taken into account. MANEC containing a well differentiated NET (Grades 1 or 2) component and an adenocarcinoma

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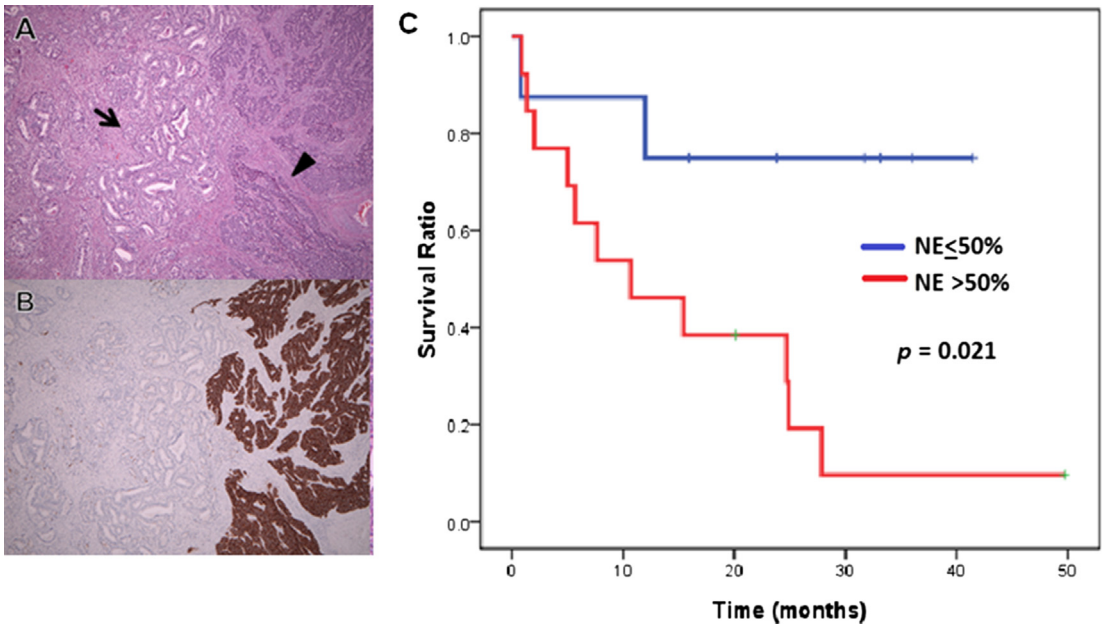

Fig. 1. (A and B) Tumor showing both adenocarcinomatous (arrow) and neuroendocrine (NE) components (arrowhead); the chromogranin stain highlights the NE-component portion of the tissue. (C) Kaplan–Meier plot of the overall survival of 21 patients with high grade MANEC, stratified by NE components in the primary tumor.

Table 2
The relationship between the pathology of tumor emboli, regional lymph node, and distant liver metastasis.

Case	NE component (%)	Ki67	Diagnosis	WHO grading of adenocarcinoma	Tumor emboli	Lymph nodes	Distant liver metastasis
1	30	—	MANEC	2	Ad	Ad	Ad
2	30	—	MANEC	2	Ad	NE	—
3	40	—	MANEC	2	NE	NE	—
4	50	—	MANEC	3	Ad + NE	Ad + NE	—
5	50	—	MANEC	2	Ad + NE	Ad + NE	—
6	60	80	MANEC	2	NE	NE	NE
7	60	—	MANEC	2	NE	NE	—
8	60	70	MANEC	1	Ad + NE	Ad + NE	—
9	60	—	MANEC	1	NE	Ad + NE	NE
10	70	—	MANEC	1	NE	NE	—
11	70	90	MANEC	3	NE	NE	—
12	70	—	MANEC	2	NE	NE	—
13	70	—	MANEC	2	NE	NE	—E
14	80	80	NEC	1	NE	—	—
15	80	—	NEC	2	NE	NE	—
16	80	90	NEC	1	—	—	—
17	90	—	NEC	2	NE	NE	—
18	95	70	NEC	2	NE	NE	—
19	10	—	Ad	2	Ad	—	—
20	15	—	Ad	2	Ad	Ad	—
21	20	—	Ad	2	NE	—	—

Ad = adenocarcinoma; MANEC = mixed adenoneuroendocrine tumor; NE = neuroendocrine tumor; NEC = neuroendocrine carcinoma.

Table 3
A high neuroendocrine component in primary mixed neuroendocrine tumors resulted in the development of a pure neuroendocrine component in tumor emboli, lymph node, or distant liver metastasis.

	Tumor emboli				Regional lymph node				Distant liver metastasis			
	NE	Ad	Both	*P*	NE	Ad	Both	*p*	NE	Ad	Both	*p*
Neuroendocrine component (%)												
≤ 50	2	4	2	0.012	2	2	2	0.065	0	1	0	0.046
> 50	11	0	1		9	0	2		3	0	0	

Ad = adenocarcinoma; NE = neuroendocrine tumor.

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component should be treated as adenocarcinoma. MANEC containing a poorly differentiated NEC (Grade 3) component should be treated as NEC,[13−15] although this recommendation lacks enough evidence to support it. In our study, 75% (3/4) of patients with high-grade malignant MANEC had a pure NE component and 25% (1/4) had a pure adenocarcinomatous component in distant liver metastases. Distant metastasis to vital organs is the main cause of death from cancer. Thus, it is reasonable to identify the tumor components in the distant metastatic tumor of patients with MANEC, which may help clinicians develop new treatment strategies.

In 1987, Lewin[2] presented a method for classifying mixed-type adenoneuroendocrine carcinomas according to three separate patterns: (1) the exocrine and endocrine areas are admixed within the same tumor mass and constitute at least one-third of the tumor; (2) concurrent NE and exocrine differentiation are exhibited by the same tumor cells, i.e., amphicrine tumors; and (3) the exocrine and endocrine areas are juxtaposed, but not admixed within the same tumor mass.[2] This study defined the criteria for determining the extent of the NE component in mixed-type adenoneuroendocrine carcinomas as 30%, which incidentally, is the same as that currently used.[2,10] However, the significance of the NE component in MANEC is unclear and has received limited clinical attention.[11] In this study, we wanted to investigate the role of NE component in these patients. Thus, we recruited an additional eight patients (3 patients with NE < 30% and 5 patients with NE > 70%) with varying NE components. We enrolled a total of 21 patients with a poorly differentiated (Grade 3) NEC component, at various percentages ranging from 10% to 90%. We found that a high NE component (> 50%) in the primary tumor was associated with poor prognosis. This phenomenon is also observed in prostatic adenocarcinomas with NE differentiation, which shows that the NE component is a negative prognostic factor.[17,18] We also found high NE component increased rate of pure NE component tumors in tumor emboli and distant liver metastases (Table 3). The tumor components of the distant metastases should be the main factor that affects tumor progression. However, it is sometimes difficult to obtain tissue samples from metastatic lesions. In this situation, the results of our study lead us to suggest that the pathology of metastatic lesions can be determined by observing the components present in tumor emboli or the percentage of the NE component in the primary tumor.

A high NE component was associated with an adverse outcome and increased rate of pure NE component in distant liver metastases. The negative prognostic value of a high NE component of high-grade gastrointestinal MANECs might be due to the clinical course of these diseases being more likely to be pure poorly differentiated NE carcinomas.[19]

Most gastrointestinal MANECs generally show nuclear immunoreactivity for CDX2. However, some reports also showed CDX2 also expressed in small number of lung NE carcinomas.[20,21] In addition, La Rosa et al[20] further subdivided colorectal MANEC into four groups according to CDX2, TTF1, and ASH1 expression. The expression of these transcription factors does not show a prognostic significance, but their expression is interesting because it indicates the phenotypical heterogeneity of the neuroendocrine component of gastrointestinal MANEC. Another interesting issue is the possible mechanisms of two different components of cancer cells coexisting in one tumor. There are two major hypotheses: these tumors might arise independently from different stem/progenitor cells, or they might derive from a common, multipotent stem/progenitor cell.[13,15] Recently, Scardoni et al[22] used next-generation sequencing to assess 54 cancer-associated genes in patients with MANECs. Both the exocrine and neuroendocrine components of six MANECs were microdissected and analyzed. They found that five of the six MANECs presented an overlapping mutational profile in both components. The result suggested two different components from the same stem/progenitor cell.

Neuron-specific enolase and chromogranin A are both biomarkers of neuroendocrine neoplasms that have potential to predict the outcome or monitor the treatment response. However, the relationship of these biomarkers in MANEC is still not clear. It warrants further studies in the future.

The main limitation of this study is its small sample size ($n = 21$). Also, MANEC is a very rare malignancy, and most patients are diagnosed with distant metastases. Both of the reasons mentioned above cause difficulties in the collection of samples for analysis. Further studies are required to validate the role of NE components in high-grade malignant MANEC.

In conclusion, we found that the extent of NE and adenocarcinomatous components in primary high-grade malignant MANECs and their metastatic tumors are not always the same. Furthermore, the NE component of tumors involved in tumor emboli was similar with that in lymph node and distant liver metastases. A high NE component in the primary tumor predicted a poor prognosis and increased the rate of pure NE component tumors in tumor emboli or distant metastases. These data may assist clinicians in deciding the appropriate treatment method for patients with high-grade malignant MANEC.

Acknowledgments

This research was supported by grants from the Yen Tjing Ling Medical Foundation (CI-103-10), and from the Taiwan Clinical Oncology Research Foundation to M.-H. Chen.

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