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Bcl-2 and Noxa are potential prognostic indicators for patients with gastroenteropancreatic neuroendocrine neoplasms

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Abstract

Purpose Bcl-2 family of proteins are of great significance in the pathogenesis and development of tumor. In this study, the relationship between the expression of Bcl-2 family proteins and clinicopathological features and prognosis of NENs were further investigated.

Methods 105 Patients diagnosed with gastroenteropancreatic NENs (GEP-NENs) with the paraffin specimen of the tumor available were retrospectively included, immunohistochemistry (IHC) was performed to detect the expression of Bcl-2 family proteins in paraffin-embedded samples. Student's t test and Chi-square test were applied to compare the difference of quantitative and categorical variables, respectively. Prognosis analysis was conducted according to Kaplan–Meier method. Univariate COX and multivariate COX regression analysis were used to screen out the independent prognostic factors.

Results The IHC score of Bcl-2 was significant higher in neuroendocrine carcinomas (NECs) patients (65.6% vs. 38.4%, P = 0.010), while higher IHC score of Noxa was more common in neuroendocrine tumors (NETs) patients (49.3% vs. 25.0%, P = 0.020). Survival analysis suggested that patients with higher Bcl-2 expression and lower Noxa expression had worse survival rate (42.9% vs. 73.2%, P < 0.001; 42.6% vs. 81.8%, P < 0.001). Multivariate cox analysis indicated that high Bcl-2 expression was an independent factor associated with poor DFS (hazard ratio [HR]: 2.661; 95% confidence interval [CI]: 1.385–5.110) and poor OS (HR: 3.205; 95% CI: 1.587–6.473; P = 0.001), while higher Noxa expression was associated with improved DFS (HR:0.324; 95% CI: 0.147–0.713) and better OS (HR: 0.293; 95% CI: 0.126–0.684).

Conclusion Higher expression of Bcl-2, and lower expression of Noxa were associated with unfavorable prognosis of GEP-NEN patients.

1. Introduction

Neuroendocrine tumor (NET) is a rare but highly heterogeneous malignant tumor, originating from neuroendocrine cells or peptide-energetic neurons, which has received increasing attention in recent years. In a series of 64,971 NETs reported by the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute, the reported annual incidence rate grew from 1.09 per 100,000 in 1973 to 6.98 per 100,000 (1). In addition to the surgical treatment, the therapeutic effects of chemotherapy and approved target drugs are very limited. For instance, the median progression-free survival (PFS) of targeted therapies such as sunitinib and everolimus in pancreatic NET were 11.4 months and 11.0 months, respectively. In non-pancreatic neuroendocrine tumors, the PFS of sunitinib and everolimus was 1.7 months and 11.0 months, respectively(2–5). The market demand for targeted drugs in the field of GEP-NENs is still huge, and the research and development of new targeted small molecule drugs is still an arduous subject that needs to be resolved urgently.

Evasion apoptosis is one of the main steps of tumor initiation and progression, Apoptosis, which is one of the important forms of cell death, is tightly regulated by the balance between the activities of proteins in the Bcl-2 family, and plays an essential role in maintaining homeostasis in adult tissues(6, 7). The imbalance between cell proliferation and apoptosis is of great importance for tumorigenesis(8). In some solid and hematologic tumors, Bcl-2 overexpression is associated with more malignant phenotypes and poor prognosis(9–11). Based on this theory, some small molecules that target Bcl-2 were designed to activate apoptosis and have been developed and tested in clinical trials, marking breakthroughs in the field of cell death. Venetoclax, for instance, has been approved by the Food and Drug Administration (FDA) for the treatment of adult chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL), as well as combined treatment with azacitidine or decitabine for acute myeloid leukemia (AML)(9,

12). Although previous studies have found that pancreatic small cell NECs has a higher Bcl-2 expression level than pancreatic NETs(13), the expression of Bcl-2 family protein in GEP-NENs is still not known yet. To this end, we aimed to further study the expression of Bcl-2 family proteins in patients with GEP-NENs and investigate their relationships with the prognosis of patients.

2. Materials And Methods

2.1. Patient cohort and data collection

Parraffin-embeded tumor tissue samples were collected from 105 patients who were diagnosed with GEP-NENs at the first affiliated hospital, Sun Yat-Sen University (Guangzhou, China) from January 2008 to November 2016. All patients included were pathologically reassessed according to the 2019 world health organization(WHO) criteria(14) by a pathologist with extensive experience and TNM staging was re-performed according to the eighth edition AJCC guidelines(15). All the clinicopathological data, including gender, age, tumor grade, tumor size, lymphatic and distant metastasis status and follow-up data, were retrospectively collected from medical record system of The First Affiliated Hospital, Sun Yat-Sen University. This study was approved by the clinical ethics committee of The First Affiliated Hospital, Sun Yat-sen University (2020489) and undertaken in accordance with the ethical standards of the World Medical Association Declaration of Helsinki.

2.2. Immunohistochemical staining

Immunohistochemistry (IHC) was carried out as followed. In brief, first, the paraffin-embeded samples were sectioned into 4μm-thick sections and dewaxed in xylene, rehydrated in rinsed graded ethanol solutions. Endogenous peroxidase activity was then blocked with 3% hydrogen peroxide solution for 10 minutes, rinsed in phosphate buffered saline (PBS) 3 times for 5 min each. Then the tissue sections were heated at 100℃ for 5 minutes in citrate (10mmol/L, pH 6.0) solution to retrieve the antigens. After cooling to room temperature, serum blocker was added to block non-specific antigen, and were incubated with the primary antibody Bcl-2 (ab182858; Abcam, Cambridge, MA, USA, 1:1000 dilution), Bcl-xL (cat#2764, Cell Signaling Technology, USA, 1:3000 dilution), Mcl-1(ab32087; Abcam, Cambridge, MA, USA, 1:200 dilution), Noxa (ab13654; Abcam, Cambridge, MA, USA, 1:2000 dilution), PUMA (ab33906; Abcam, Cambridge, MA, USA, 1:200 dilution) at 4℃ overnight, followed by washing with PBS for three times, biotinylated goat anti-mouse IgG (1:400; Sigma, St. Louis, MO, USA) or goat anti-rabbit IgG (1:400; Sigma) for 30 min at room temperature. Finally, the signal was developed for visualization with 3,3’-diaminobenzidine tetrahydrochloride (DAB, DAKO, GLOSTRUP, Denmark), and all of the slides were counterstained with hematoxylin.

2.3. Immunohistochemical analysis

The slides were evaluated independently by two observers blinded to clinicopathological information. Any disagreement was resolved by a jointly reevaluation. Expression of markers was evaluated by the percentage of positive cells and staining intensity. The percentage of positive cells was evaluated quantitatively and scored as 0 for staining of < 2% of total cells counted, 1 for staining of 2–25%, 2 for staining of 26–50%, 3 for staining of 51–75%, and 4 for staining of > 75% of the cells examined. Intensity was graded as follows: 0, negative staining; 1, weak; 2, moderate; and 3, strong staining. A total “staining score” of 0–12 was calculated by multiplying staining intensity score and staining percentage score(16, 17).

2.4. Follow-up

All patients were followed up every three to six months for the first two years after diagnosis, then annually after two years, with the last telephone follow-up in June 2020. The time of death was recorded for patients who died, and the last follow-up date and status of patients who could not be reached were obtained from the hospital system. The

primary outcome was patient overall survival (OS), defined as the time from the date of diagnosis to death or the last follow-up. The secondary outcome of the study was disease-free survival (DFS), defined as the time from the date of diagnosis to disease recurrence or death or the last follow-up.

2.5. Statistical analyses

IBM SPSS software, version 25.0 (IBM, Chicago, IL, USA) was used for statistical tests. The cut-off value of markers expression was determined by the median IHC score as previous study(18, 19). The IHC score which was greater than cut-off value was defined as high expression, while lower than cut-off value was defined as low expression. Quantitative variables were presented as mean ± average and categorical variables were presented as percentages. Chi-square test (or Fisher's exact test) was applied to compare the categorical variables. Cox proportional hazards regression was used for univariate analyses and factors, for which $P < 0.05$ in univariate survival analyses, were further assessed in multivariate Cox models. Results were presented with hazard ratio (HR) and 95% confidence intervals (CI). Survival analyses were performed via Kaplan-Meier method and long-rank test was used to assess the individual risk factors related with survival. Two sides P-value < 0.05 was considered statistically significant.

3. Results

3.1. Clinicopathological characteristics of patients

In total, 105 patients with GEP-NENs were included, including 28 cases (26.7%) with primary tumors in the stomach, 39 cases (37.1%) in the gut, and 38 cases (36.2%) in the pancreas (see Table 1). 63 (60.0%) of the patients were male and 42 (40.0%) were female with an average age of 51.0 years old (range, 10–81 years). As for tumor grade, 33 (31.4%) patients were diagnosed with grade 1 (G1), 27 (25.7%) with grade 2 (G2), 13 (12.4%) with grade 3 (G3), and 32 (30.5%) with NECs. Among these patients, 61(48.6%) of patients had lymph node metastases while 49 (46.7%) of patients had distant metastases.

Table 1
Clinical data of 105 patients of GEP-NEN

Characteristic	Total(n = 105)
Sex, n (%)	
Male	63(60.0)
Female	42(40.0)
Age ($\bar{x} \pm S$)	51.0 ± 14.7
≤ 60	81(77.1)
> 60	24(22.9)
Primary tumor location, n (%)	
Stomach	28(26.7)
Gut	39(37.1)
Pancreas	38(36.2)
Grade, n (%)	
G1	33(31.4)
G2	27(25.7)
G3	13(12.4)
NEC	32(30.5)
T stage	
X	5(4.8)
1	29(27.6)
2	19(18.1)
3	27(25.7)
4	25(23.8)
N stage	
0	54(51.4)
1	45(42.9)
2	6(5.7)
M stage	
0	56(53.3)
1	49(46.7)
Surgical operation	
Yes	24(22.9)

Characteristic	Total(n = 105)
No	81(77.1)

3.2. Correlation analysis between Bcl-2 family markers and clinicopathological characteristics

As shown in Fig. 1, immunohistochemical staining for Bcl-2and Noxa were found in cytoplasm of tumor cells. High expression of Bcl-2 was found in 49 (46.7%) tumor samples, while high expression of Noxa was found in 44 (41.9%) tumor samples. In order to determine the relationship between the expression of Bcl-2 family markers and clinicopathological parameters, we then took the Bcl-2 family markers expressions and clinical characteristics (including age, sex, primary tumor location, WHO grade, tumor stage, node stage, metastasis stage, survival status) into Chi-square test analysis (see Table 2). High expression of Bcl-2 mostly occurs in NEC patients (65.6% vs. 38.4%, P = 0.010) and associated with worse prognosis (65.1% vs. 33.9%, P = 0.002), while Bcl-2 expression and the other clinical parameters did not differ (P > 0.05). However, a diametrically opposite result was witnessed in Noxa. High expression of Noxa mostly occurs in NET patients (49.3% vs. 25.0%, P = 0.020) and associated with better prognosis (58.1% vs. 18.6%, P < 0.001). In addition, high Noxa expression seems to be associated with younger age at diagnosed (48.1% vs. 20.8%, P = 0.017). Expressions of Mcl-1, Bcl-xL, PUMA were not significantly different in subgroups of clinicopathological characteristics and survival outcomes (see Supplementary Table 1).

Table 2
relationship between the expression of Bcl-2, Nox-a and clinicopathologic parameters

Parameters	Expression of Bcl-2		P value*	Expression of Nox-a		P value*
	Low (n = 56, %)	High (n = 49, %)		Low (n = 61,%)	High (n = 44, %)	
Age,year						
≤60	45(55.6)	36(44.4)	0.402	42(51.9)	39(48.1)	0.017
>60	11(45.8)	9(54.2)		19(79.2)	5(20.8)	
Sex						
Male	34(54.9)	29(46.0)	0.873	40(63.5)	23(36.5)	0.170
Female	22(52.4)	20(47.6)		21(50.0)	21(50.0)	
Primary tumor location						
Stomache	12(42.9)	16(57.1)	0.409	21(75.0)	7(25.0)	0.024
Gut	23(59.0)	16(41.0)		24(61.5)	15(38.5)	
Pancreas	21(55.3)	17(44.7)		16(42.1)	22(57.9)	
WHO Grade						
NET	45(61.6)	28(38.4)	0.010	37(50.7)	36(49.3)	0.020
NEC	11(34.4)	21(65.6)		24(75.0)	8(25.0)	
T stage						
1–3	41(54.7)	34(45.3)	0.204	41(54.7)	34(45.3)	0.242
4	10(40.0)	15(60.0)		17(68.0)	8(32.0)	
N stage						
0	32(59.3)	22(40.7)	0.210	27(50.0)	27(50.0)	0.084
1–3	24(47.1)	27(52.9)		34(66.7)	17(33.3)	
M stage						
0	32(57.1)	24(42.9)	0.403	30(53.6)	26(46.4)	0.315
1	24(49.0)	25(51.0)		31(63.3)	18(36.7)	
Survival status						
Alive	41(66.1)	21(33.9)	0.002	26(41.9)	36(58.1)	< 0.001
Dead	15(34.9)	28(65.1)		35(81.4)	8(18.6)	
Note: * X^2test, a, Pearson's Chi-Square test						

3.3. Bcl-2 and Noxa are potential predictors for survival outcome of patients with GEP-NENs

In total, 105 patients were followed up for a median of 45 months (range 1-129 months). During the follow-up, 43 (41.0%) patients died, of whom 3 (7.0%) cases were classified as G1, 6 (14.0%) cases as G2, 8 (18.6%) cases as G3, and 26 (60.5%) cases as NECs. Among these patients, 41 (95.3%) patients died due to tumor progression, 1 (2.3%) patient died due to cerebrovascular disease, and 1 (2.3%) patient died by accident. To evaluate the expression of Bcl-2 family markers as predictors for prognosis, Kaplan– Meier survival curves were used for analysis. The high level of Bcl-2 is significantly correlated with poor DFS ($p < 0.001$, Fig. 2A), we then divided the patients into NETs and NECs subgroups for further analysis and found that the high expression of Bcl-2 has a predictive effect only in NET patients ($p = 0.032$, Fig. 2B) but not in NEC patients ($p = 0.090$, Fig. 2C). A similar trend was observed in OS, high Bcl-2 expressions are correlated with poor OS ($p < 0.001$, Fig. 3A), no matter in NET patients ($p = 0.012$, Fig. 3B) or NEC patients. ($p = 0.032$, Fig. 3C). On the contrary, Noxa can be a positive prognostic factor for DFS ($p < 0.001$, Fig. 2D) and OS ($p < 0.001$, Fig. 3D), when dividing patients into NET and NEC subgroups, Noxa can also be a predictive marker with positive effect, but for NEC group patients, there is no difference between high and low Noxa expression for DFS ($p = 0.071$, Fig. 2F). Other markers were also subjected to long-rank test, the expressions of Bcl-xl, Mcl-1, and PUMA were not correlated with DFS (S-Figure1) and OS (S-Figure2).

3.4 Age, primary tumor location, metastatic stage, Bcl-2 expression and Noxa expression could be a risk model of DFS and OS for NEN patients

As shown in Table 3, univariate and multivariate Cox regression analyses were used to identify risk factors for NEN patients. Univariate Cox regression analyses demonstrate that age above 60 years old (HR: 3.269; 95% CI: 1.816–5.883, $p < 0.001$), NEC (HR: 5.858; 95% CI: 3.298–10.402, $p < 0.001$), T stage 4 (HR: 2.158; 95% CI: 1.187–3.9214, $p = 0.012$), node metastasis 1–2 (HR: 1.806; 95% CI: 1.025–3.183, $p = 0.041$), distance metastasis (HR: 2.278; 95% CI: 1.283–4.043, $p = 0.005$), and high Bcl-2 expression (HR: 2.949; 95% CI: 1.643–5.295, $p < 0.001$) were negatively and significantly correlated with DFS, while pancreas NENs (HR: 0.364; 95% CI: 0.179–0.741, $p = 0.005$), operation (HR: 0.326; 95% CI: 0.183–0.581, $p < 0.001$), high Noxa expression (HR: 0.293; 95% CI: 0.152–0.563, $p < 0.001$) were positively correlated with DFS. Multivariate Cox regression analyses indicated that NEC (HR: 8.899; 95% CI: 3.681–21.510, $p < 0.001$), node metastases 1–2 (HR: 0.481; 95% CI: 0.241–0.958, $p = 0.037$), distant metastases (HR: 2.260; 95% CI: 1.120–4.562, $p = 0.023$), operation (HR: 0.310; 95% CI: 0.140–0.690, $p = 0.004$), high Bcl-2 expression (HR: 2.661; 95% CI: 1.385–5.110, $p = 0.003$), high Noxa expression (HR: 0.324; 95% CI: 0.147–0.713, $p = 0.005$) were independent predictors of NEN patients' DFS.

Table 3
Univariate and multivariate Cox regression analysis of overall survival and disease-free survival

Variable	HR(95%CI)	P	HR(95%CI)	P	HR(95%CI)	P	HR(95%CI)	P
Age (≤ 60 year vs. >60 year)	3.269(1.816–5.883)	< 0.001	1.056(0.466–2.390)	0.896	3.631(1.947–6.772)	< 0.001	1.309(0.579–2.957)	0.518
Sex (Male vs. Female)	0.882(0.498–1.562)	0.667			0.844(0.455–1.567)	0.591		
Primary tumor location (stomach vs. gut)	0.602(0.316–1.149)	0.124	1.535(0.745–3.165)	0.246	2.627(1.248–5.531)	0.011	0.428(0.156–1.178)	0.100
Primary tumor location (stomach vs. pancreas)	0.364(0.179–0.741)	0.005	1.666(0.624–4.446)	0.308	1.380(0.637–2.989)	0.663	0.559(0.221–1.413)	0.219
WHO Grade (NET vs. NEC)	5.858(3.298–10.402)	< 0.001	8.899(3.681–21.510)	< 0.001	6.507(3.481–12.163)	< 0.001	8.860(3.958–24.560)	< 0.001
T (1–3 vs. 4)	2.158(1.187–3.921)	0.012	1.399(0.666–2.937)	0.375	1.895(0.992–3.619)	0.053		
N (0 vs.1–2)	1.806(1.025–3.183)	0.041	0.481(0.241–0.958)	0.037	1.646 (0.898–3.018)	0.107		
M (0 vs. 1)	2.278(1.283–4.043)	0.005	2.260(1.120–4.562)	0.023	2.023(1.095–3.736)	0.024	1.583(0.804–3.118)	0.184
Operation (No vs. Yes)	0.326(0.183–0.581)	< 0.001	0.310(0.140–0.690)	0.004	0.322(0.174–0.597)	< 0.001	0.314 (0.142–0.696)	0.004
Bcl-2 expression (low vs. high)	2.949(1.643–5.295)	< 0.001	2.661(1.385–5.110)	0.003	3.669(1.914–7.032)	< 0.001	3.205(1.587–6.473)	0.001
Noxa expression (low vs. high)	0.293(0.152–0.563)	< 0.001	0.324(0.147–0.713)	0.005	0.216(0.099–0.469)	< 0.001	0.293(0.126–0.684)	0.005
Bcl-xl expression (low vs. high)	0.860(0.476–1.555)	0.618			0.912(0.483–1.723)	0.777		

Abbreviations: HR, hazard ratio; 95%CI, 95% confidence interval.

Puma expression (low vs. high)	0.758(0.430–1.336)	0.338			0.823 (0.449–1.511)	0.530		
Mcl-1 expression (low vs. high)	0.866(0.494–1.519)	0.615			0.720(0.388–1.337)	0.299		
For patients diagnosed as NET								
Bcl-2 expression (low vs. high)	2.459(1.052–5.748)	0.038	3.497(1.399–8.739)	0.007	3.272(1.231–8.701)	0.017	4.189(1.494–11.745)	0.006
Nox-a expression (low vs. high)	0.182(0.061–0.539)	0.002	0.144(0.047–0.442)	0.001	0.184(0.053–0.640)	0.008	0.151(0.042–0.541)	0.004
For patients diagnosed as NEC								
Bcl-2 expression (low vs. high)	2.014(0.876–4.629)	0.099	1.992(0.864–4.593)	0.106	2.514(1.042–6.607)	0.040	2.355(0.972–5.703)	0.058
Nox-a expression (low vs. high)								
Abbreviations: HR, hazard ratio; 95%CI, 95% confidence interval.								

As for OS, the univariate analysis revealed that age above 60 years old (HR: 1.309; 95% CI: 1.947–6.772, $p < 0.001$), tumor located in gut (HR: 2.627; 95% CI: 1.248–5.531, $p = 0.011$), NEC (HR: 6.507; 95% CI: 3.481–12.163, $p < 0.001$), distant metastases (HR: 2.023; 95% CI: 1.095–3.736, $p = 0.024$), operation (HR: 0.322; 95% CI: 0.174–0.597, $p < 0.001$), high Bcl-2 expression (HR: 3.669; 95% CI: 1.914–7.032, $p < 0.001$), high Noxa expression (HR: 0,216; 95% CI: 0.099–0.469, $p < 0.001$) were significant correlated with OS. We further carried out these factors for multivariate Cox regression analyses, which showed that NEC (HR: 8.860; 95% CI: 3.958–24.560, $p < 0.001$), operation (HR: 0.314; 95% CI: 0.142–0.696, $p = 0.004$), and high Noxa expression (HR: 0.293; 95% CI: 0.126–0.684, $p = 0.005$) were identified as independent predictors of GEP-NEN patients' OS.

4. Discussion

In order to further explore biomarkers to predict long-term survival in GEP-NENs, our study evaluated the Bcl-2 family protein expressions in tumor tissue of GEP-NEN patients. There are considerable evidences to suggest that Bcl-2 and Noxa expressions correlate with tumor grade and survival prognosis. That is, overexpression of Bcl-2 protein and low expression of Noxa protein indicated poor tumor differentiation and poor prognosis.

Bcl-2 is the fundamental member of Bcl-2 family of apoptosis and classified as oncogene(20). Dysregulation of bcl-2 family proteins has been found in a variety of tumors such as lung cancer, melanoma, and AML(21–23). In this study, we found that the expression of Bcl-2 in NECs was significantly higher than that of NETs in GEP-NENs, and the same trend was also observed in previous studies which focused on pancreatic NENs, suggesting that overexpression of Bcl-2 may be responsible for higher proliferation rate and more malignant phenotype of NECs compared to NETs(13). This phenomenon was also observed in NETs derived from lung, that is, the expression of Bcl-2 in small cell lung cancer (SCLC) was higher than that of typical carcinoid (TC) and atypical carcinoid (AC). What's more, considerable evidence suggest that a positive correlation was observed between Bcl-2 and chromogranin A (CgA), which indicated the expression of Bcl-2 may be involved in neuroendocrine differentiation(13, 21, 24). In addition, overexpression of Bcl-2 also induces resistance of chemotherapy and targeted therapies(25, 26). Currently, several Bcl-2 inhibitors, including Venetoclax, have demonstrated a marked activity *in vitro* studies and good pharmacological effects in clinical trials and have been approved by FDA for the treatment of SCLC, AML and chronic CLL(27–29). We found overexpression of Bcl-2 in poorly differentiated GEP-NECs, suggesting that inhibitors targeting Bcl-2 may also be a future option for these patients.

On the contrary, Noxa, which selectively binds to Mcl-1, belongs to a subclass of BH3-only proteins and plays pro-apoptotic effect through the neutralization of Mcl-1(30, 31). Previous studies have revealed that Noxa gene expression and protein function have been linked to cell death in kinds of hematopoietic and solid cancers, such as melanoma, multiple myeloma (MM), and CLL(31–33). In melanoma and breast cancer studies, induced upregulation of Noxa enhances the pharmacological effects of BH3 analogue ABT-737(34, 35). In this study, we also observed that the expression of Noxa is significantly higher in NETs, compared with NECs, and was positively associated with prognosis. Restoring or enhancing Noxa expression may significantly increase treatment efficacy and may serve as a worthwhile strategy to be explored in GEP-NENs.

This study still has some limitations. First of all, this is a retrospective study, with inherent limitations of retrospective research, such as missing some data. Secondly, given to limitations in the understanding of NENs in the early years, some patients did not receive standard treatment. Consequently, further large sample and multi-center studies are of great importance to validate these conclusions.

Conclusion

Taken together, our study, for the first time, systematically detected the expression of Bcl-2 family proteins in GEP-NENs, and further evaluated the relationship between the expression of Bcl-2 family proteins and the prognosis of GEP-NEN patients. Our results demonstrated that Bcl-2 and Noxa were valuable and independent prognostic indicators of DFS and OS in GEP-NENs. It gave us a new understanding of NENs and laid the ground for the application of drugs targeting Bcl-2 family proteins in treating NENs.

Declarations

Author contributions

All authors contributed to the study conception and design. Data collection and analysis were performed by Guo Yu, Zhang Lin and Zhang Ning; Experiments design: Yang Dajun and Chen Jie; Immunohistochemical staining and analysis: Liu Man, Luo Qiuyun;

Manuscript writing: Guo Yu, Zhang Lin and Zhang Ning, and Liu Man. All authors read and approved the final manuscript.

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Conflict of Interest Disclosure Statement

The authors have declared that no competing interest exists.

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Figures

Figure 1

IHC staining for Bcl-2 and Noxa. A. High and low expression of Bcl-2 protein (200x). B. High and low expression of Noxa protein (200x). Second line shows a higher magnification(400x), respectively.

Figure 2

Kaplan-Meier analysis showing high Bcl-2 expression is associated with poor DFS (A), When patients were grouped by NET and NEC, this relationship existed only in the NET group (B), but not in the NEC group (C). On the contrary, high Noxa expression is associated with better DFS (D), and this relationship is still existed only in NET group (E) not in NEC group (F).

Figure 3

Kaplan-Meier analysis showing high Bcl-2 expression is associated with poor OS (A). When patients were grouped by NET and NEC, this relationship existed in the NET group (B), and NEC group (C). On the contrary, high Noxa expression is associated with better OS (D), and this relationship is still existed not only in NET group (E) but also in NEC group (F).

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