Association of γ-aminobutyric acid type A receptor-associated protein with prognosis in patients after radical pancreatic cancer treatment

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Research article

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Abstract

Background Pancreatic cancer is difficult to cure and many factors influence patient prognosis, of which autophagy is a recent research hotspot, and GABARAP plays a key role in autophagy, which has been found to interfere with cancer cell survival and metastasis in a variety of tumours. In this study, we analysed the correlation between GABARAP and patient prognosis in pancreatic cancer, as well as its correlation with clinicopathological parameters and its impact on the efficacy of chemotherapy in pancreatic cancer patients.

Methods: The pancreatic tissues of 76 pancreatic cancer patients after R0 resection were screened according to the criteria, and the expression levels of GABARAP were determined by using immunohistochemistry (MaxVision) to label the pancreatic cancer tissues and normal pancreatic tissue around carcinoma in these two types of specimens, and the relationship between GABARAP and other factors and the disease-free and overall survival of patients after radical pancreatic cancer treatment was evaluated by single factor survival analysis and Cox regression analysis.

Results The expression ratio of GABARAP in pancreatic cancer tissue was drastically higher than that in normal pancreatic tissue adjacent to cancer. Cox regression model evaluation showed that GABARAP and postoperative adjuvant chemotherapy were the overall survival of patients after radical pancreatic cancer Period independent prognostic indicators and both are protective factors for the prognosis of pancreatic cancer patients. Further data analysis found that postoperative chemotherapy drastically increased the total patient Survival period in GABARAP-negative patients but had no drastically effect on the patient's relapse-free survival period.

Conclusion: The expression of GABARAP in pancreatic cancer tissues was drastically up-regulated, and patients with high expression of GABARAP in pancreatic cancer tissues had better prognosis, but had no drastically effect on the relapse-free survival of patients after radical operation of pancreatic cancer. The expression of GABARAP in pancreatic cancer tissues and Postoperative adjuvant chemotherapy is an independent indicator of patients' prognosis after radical pancreatic cancer resection. Both are protective factors. The high expression of GABARAP in pancreatic cancer may indicate that the adjuvant chemotherapy is low benefit.

Background

Pancreatic malignancy is a common malignancy known for its difficulty in detection, low resectable rate and high mortality rate, and although pancreatic cancer is statistically ranked 8th in incidence, it is ranked 5th in mortality[1, 2], Only 2%-9% have a 5-year survival rate[3], This is firstly because there is a lack of effective screening methods to detect the disease. Second, the almost complete absence of symptoms associated with early pancreatic cancer, coupled with the abundance of blood vessels and lymphatic vessels in pancreatic tissues, thin peritoneal membrane and easy metastasis, resulting in the majority of pancreatic cancer patients were already stage IV tumors at the time of diagnosis, and only 20% of them

were clinically viable for surgery[4]. However, the prognosis of patients with pancreatic cancer that can be treated radically remains poor, with 5-year survival rates ranging from 13% to 25% in patients who are completely resected[5], Many scholars look for factors that affect the prognosis of pancreatic cancer patients after radical surgery and find that most pancreatic cancer chemotherapy is associated with poor outcomes[6] .

In recent years, many scholars have found a strong correlation between autophagy and the treatment and prognosis of pancreatic cancer. Pardo et al. [7] found that gemcitabine was shown to induce vesicle membrane protein 1 (VMP1)-mediated autophagy and lead to apoptosis in pancreatic cancer cells in a pancreatic cancer cell line. Cannabinoids inhibit cell growth by inducing reactive oxygen species-mediated autophagic death, thereby sensitizing drug-resistant pancreatic cancer cells to gemcitabine[8]. Gemcitabine or 5-FU may enhance autophagy, modulate chemotherapy tolerance, and induce pancreatic cancer cell death in combination with omeprazole[9].

The human GABARAP gene is located on the posterior strand of chromosome 17, 17p13.1, with a gene size of 1.5kb. The gene encodes a GABA(A) receptor-associated protein and is expressed in the human pancreas at a median gene level（RPKM=38.499±4.242）https://www.ncbi.nlm.nih.gov/gene/11337）. GABA(A) receptor-associated protein（GABARAP） is a 14kDa-sized cytoplasmic protein that is highly positively charged at its N terminus and shares sequence similarity with light chain 3 of microtubule-associated proteins 1A and 1B.[10], GABA(A) receptors can be involved in the aggregation of GABA(A) receptors on the cytoskeleton through mediated interactions with the cytoskeleton, and are also involved in autophagy. In addition, it has been shown that GABARAP is a tumor suppressor gene. The mRNA and protein levels of GABARAP are lower in primary breast cancer tissues compared to normal breast tissues, and ectopic expression of the GABARAP gene in low-expressing breast cancer cell lines reduces tumor growth rate[11]. This tumor suppressive property of GABARAP can be mediated through direct effects on autophagy and indirectly through the control of the translocation of receptors such as epidermal growth factor (EGFR)[12, 13]. In addition, in colorectal cancer, GABARAP is considered a useful marker of metastasis and poor prognosis[14].

Overall, the correlation between GABARAP and pancreatic cancer metastasis and prognosis suggests that GABARAP may influence tumor progression through autophagy. However, in pancreatic cancer, the correlation between GABARAP and pancreatic cancer metastasis and prognosis has not been widely reported. In this study, we verified the above hypothesis whether GABARAP is associated with pancreatic cancer metastasis and prognosis.

Method

I. Material

1. Source of cases

Select pancreatic cancer patients from Huadong Hospital Affiliated to Fudan University, which were handled by skilled professor at the Pancreatic Surgery Center, and their post-operative specimens from radical resection were collected from January 1, 2016 to December 31, 2018 in the pathology department, and the pathology of the specimens was pancreatic ductal adenocarcinoma. The inclusion criteria were patients with radical pancreatic cancer resection, negative margins, no neoadjuvant chemotherapy before surgery, pathology of pancreatic ductal adenocarcinoma and non-peripheral death, and 76 patients were finally enrolled. The postoperative complications were cured and discharged from hospital. There were 50 cases of male and 26 cases of female, aged between 29~84 years, with a median age of 65.7±9.6 years and a median age of 66.5 years. The tumors were located at the head of the pancreas in 48 cases and at the tail of the pancreas in 28 cases, with radical pancreaticoduodenectomy and radical pancreatic tail plus splenectomy respectively. The pathologic classification was pancreatic ductal adenocarcinoma with 1 case of high differentiation, 63 cases of medium differentiation and 12 cases of low differentiation. According to the American Cancer Society (AJCC) criteria, the cases in this group were clinically staged: 6 cases of stage I, 57 cases of stage II, and 13 cases of stage III. There were 19 cases of vascular invasion. 56 cases of nerve invasion. 41 cases of lymph node metastasis. Preoperative CA19-9 was increased in 68 cases. There were 53 cases of open surgery and 23 cases of minimally invasive surgery. Postoperative adjuvant chemotherapy was given in 60 cases, which include gemcitabine (GEM) alone or in combination with fluorouracil analogs such as capecitabine, tegio, and 5-fluorouracil (5-FU)/formyltetrahydrofolate. Most of the patients were reviewed regularly every 3~6 months after surgery, and postoperative follow-up was conducted by telephone, WeChat or outpatient. The following variables were selected for analysis, including sex, age (<65 years or ≥65 years), tumor location, tumor stage, pathological classification, maximum tumor diameter, lymph node metastasis, vascular invasion, nerve invasion, preoperative CA19-9 level (≤37U/ml or >37U/ml), surgical method (open or minimally invasive), postoperative adjuvant chemotherapy and GABARAP expression.

2. Antibodies and reagents

a. GABARAP mouse polyclonal antibody (NOVUS, USA) at a dilution of 1:200
b. TS-0831 Dewaxing Solution (Fuzhou Maixin Biotechnology Development Co., Ltd.)
c. TW-0821 buffer (Fuzhou Maixin Biotechnology Development Co., Ltd.)
d. TT-0801 DAB dyeing solution (Fuzhou Maixin Biotechnology Development Co., Ltd.)
e. DNS-0811 Immunohistochemical Antigen Repair Buffer (Fuzhou Maixin Biotechnology Development Co., Ltd.)

3. Major instruments

a. 4°C refrigerator (China Haier refrigerator)
b. Paraffin slicer (Leica, Germany)

c. Pathological tissue bleaching and drying instrument (Changzhou Zhongwei Electronic Instrument Co., Ltd.)
d. Electrothermal incubator 65 ℃ (Shanghai Jinghong Experimental Equipment Co., Ltd.)
e. Automatic immunohistochemical stainer (Fuzhou Maixin Biotechnology Development Co., Ltd.)
f. NIKON Y-THR (NIKON, Japan).

Method

1 Immunohistochemical staining (MaxVision).

1. According to the pathology file, select the wax blocks containing tumor tissue and the wax blocks containing normal pancreatic tissue, slice them separately, line them with HE stain, observe the presence of tumor tissue and normal pancreatic tissue under the microscope, and select the wax blocks with clear boundaries between tumor tissue and non-tumor tissue and rich tissue.
2. Slice the wax pieces and put the wax pieces gently into the hot water of a drift oven, make them float, scald them flat, then attach the wax pieces to the slides, wait for the water to dry, and put them in a 65℃-electric oven for 1 hour.
3. Select the Lomita's automatic immunohistochemistry stainer with the staining program in the computer and print out the label with the label printer, which corresponds to the antibody template to be performed.
4. Place the printed label on one end of the finished slide and place the slide on the staining rack of the stainer.
5. Place the kit containing the dewaxing solution, primary antibody, secondary antibody and DAB on the rack.
6. Place the antigen repair solution, buffer, alcohol, and distilled water in the appropriate container of the instrument.
7. Enter the operating software of the instrument, scan the section label and reagent label, then the automatic immunohistochemistry stainer will complete the steps of dewaxing, hydration, repair, add primary antibody, add secondary antibody, DAB color development, etc., and remove the slide after operation.
8. Remove the slides and re-stain with hematoxylin: rinse with water, stain with hematoxylin for 10~30 seconds, and return to blue with PBS solution, ammonia or tap water.
9. Dehydration: put into 85% ethanol, 95% ethanol and anhydrous ethanol, soak for 3 minutes each.
10. Transparency: using xylene for transparency
11. Sealing: Sealing with a neutral resin
12. Mirror examination.

2、Determination of a positive result

The positive control was a positive slide for known pancreatic cancer and the negative control was a TW-0821 buffer instead of GABARAP mouse polyclonal antibody. The negative control was TW-0821 buffer instead of GABARAP mouse polyclonal antibody. The pancreatic cancer or paraneoplastic pancreatic cell cytoplasm was found to be positive for GABARAP. The section was observed under 400 times microscope, combined with cell staining intensity analysis and calculated the ratio of the number of brown positive cells to the number of all cells in the visual field, at least 10 visual fields were calculated, and the comprehensive ratio > 25% was determined to be positive.

3、Statistical analysis

All the data in this study were analyzed using IBM SPSS Statistics 26 statistical software. The percentage of utilization and the mean ± standard deviation ($\bar{\chi}\pm s$) was expressed as count and measure data, respectively. Kaplan-Meier one-factor analysis, Log-Rank test, Landmark analysis, Empower Stats, and multifactorial analysis were performed using time-based Cox regression with $P<0.05$ as the standard for statistical significance.

Results

1. GABARAP expression and its association with various clinicopathological factors in patients

The positive rate of GABARAP in pancreatic cancer specimens was 55.26% (42/76) in 42 out of 76 patients, mainly expressed in the cytoplasm of pancreatic cancer cells, while the positive rate was 21.42% (12/76) in 12 cases in the control group of normal pancreatic tissue around carcinoma (Figure 1 and Table 2). The positive expression rate of GABARAP was significantly higher in pancreatic cancer tissues than in normal pancreatic tissue around carcinoma (P＜0.001). The cardinal correlation test (Table 1) found that GABARAP expression was not significantly correlated with sex, age (<65 years or ≥65 years), tumor location, TNM stage, pathological grade, maximum tumor diameter (<3cm or ≥3cm), lymphatic invasion, vascular invasion, nerve invasion, preoperative CA19-9 level (>37U/ml), postoperative chemotherapy ($P>0.05$).

2. Disease free survival and prognosis of patients after radical pancreatic cancer treatment

A total of 76 patients undergoing radical treatment for pancreatic cancer were included in this study, and 27 patients died as of December 31, 2019, with 1 case missing and 48 survivors. Recurrence was found in 41 patients according to the changes of type-b ultrasonic, tumor index and CT, and the site of recurrence was found by CT: local recurrence accounted for 17.1% (7/41), distant metastasis accounted for 78.0% (32/41), local recurrence with distant metastasis accounted for 4.9% (2/41), see Fig. 2. Patients had a median relapse-free survival of 12.8 months and the cumulative survival analysis is shown in Fig. 3. The relapse-free survival is shown in Fig. 4.

3. Relationship between GABARAP, clinicopathological factors and disease-free survival and overall survival in patients after radical pancreatic cancer treatment

The median disease-free survival was 12.9 months and the median overall survival was 22.0 months in 34 patients with GABARAP-positive pancreatic cancer, and the median disease-free survival was 11.8 months and the median overall survival was 17.4 months in 34 patients with negative GABARAP. One-factor K-M survival analysis showed that the GABARAP expression level was not significantly related to the disease-free survival of the patients. However, GABARAP expression level (*P*=0.015, Figure 5) and tumor site (*P*=0.018, Figure 6) were significantly associated with the overall survival of patients after pancreatic cancer radical treatment, and the overall survival of GABARAP-negative patients was significantly lower than GABARAP-positive patients. The results of one-factor K-M survival analysis were as follows (Table 3): age (*P*=0.048, Figure 7) and postoperative adjuvant chemotherapy (*P*=0.018, Figure 8) were also significantly associated with the overall survival of patients after radical pancreatic cancer treatment, while sex, TNM stage, maximum tumor diameter, vascular invasion, nerve invasion, lymph node invasion, degree of pathological differentiation, surgical modality and normal preoperative CA19-9 were not significantly associated with the overall survival of patients after radical pancreatic cancer treatment.

Among 42 patients with GABARAP-positive pancreatic cancer tissues, one-factor survival analysis showed no significant difference between clinicopathological factors and overall survival between chemotherapy and non-chemotherapy groups (*P* > 0.05), while among 34 patients with GABARAP-negative cancer tissues, one-factor analysis showed that tumor site (*P*=0.044, Figure 9) and adjuvant chemotherapy (*P*=0.005, Figure 10) were significantly associated with overall survival, but not with disease-free survival..

4Results of Cox regression multifactor analysis

In the one-factor analysis of total survival, the survival curves of GABARAP were crossed recently between tumor site and cancer tissue. The time-dependent covariates were also calculated by Cox regression: the time-dependent covariates of GABARAP for tumor site and cancer tissue were Wald card-square = 0.314, *P*=0.575; Wald card-square = 0.297, *P*=0.586, both of them *P*> 0.05, indicating that the variables did not change with time, which satisfied the Cox regression model. Cox regression multivariate analysis results (Table 4) suggest that GABARAP (*P*=0.044, Figure 11) and postoperative adjuvant chemotherapy (*P*=0.038, Figure 12) are independent prognosis of overall survival for patients after radical pancreatic cancer the indexes and the OR values of both are less than 1, suggesting that postoperative adjuvant chemotherapy and GABARAP expression are protective factors for the prognosis of pancreatic cancer patients. However, age and tumor location have no significant effect on patients after radical resection of pancreatic cancer.

Among 34 GABARAP-negative patients, Cox regression analysis showed (Table 5) that postoperative chemotherapy was associated with patients' overall survival (*P*=0.041, Figure 13) but not tumor site,

suggesting that in GABARAP-negative patients, postoperative chemotherapy significantly improved overall survival.

Discussion

GABARAP is considered to be a tumor metastasis suppressor gene in breast cancer [11], and this tumor suppressive property of GABARAP can be mediated through direct effects on autophagy and indirectly through the control of the translocation of receptors such as epidermal growth factor (EGFR)[12, 13]. Manent J et al.[15] also found that lower expression of GABARAP and VAMP2 correlates with worse prognosis in KRAS-G12-positive pancreatic adenocarcinoma. In our study, we found that the expression of GABARAP was significantly higher in pancreatic cancer tissues than in pancreatic cancer paracrine tissues. In addition, Kaplan-Meier survival analysis showed that patients with pancreatic cancer with positive expression of GABARAP survived significantly longer than patients with pancreatic cancer with negative expression of GABARAP. These findings suggest that downregulation or loss of regulation of GABARAP should promote pancreatic cancer progression and metastasis, which is consistent with previous studies[11, 15]. It suggests that GABARAP should be considered as a valuable biomarker for pancreatic cancer. The multifactorial Cox model analysis of this study showed that GABARAP, postoperative adjuvant chemotherapy are independent factors that influence the prognosis of pancreatic cancer patients. The most common causes of cancer-related death in pancreatic cancer are metastasis and recurrence. Therefore, our results suggest that GABARAP can be used as a reliable biomarker for pancreatic cancer, especially in predicting progression, metastasis, and prognosis.

Autophagy is the cell in the lack of nutrition, hormonal stimulation, oxygen deficiency, microbial invasion and environmental temperature changes and other external stimuli, or internal disorders such as organelle damage, abnormal protein accumulation, used to remove the cell's own harmful substances, synthetic proteins and production of ATP, in order to maintain intracellular homeostasis and improve cell survival. At the same time, autophagy is also a cell death mechanism, over-activated autophagy can lead to programmed cell death (also called type II programmed death), which is another programmed cell death pathway in addition to apoptosis. This is another programmed cell death pathway in addition to apoptosis. Autophagy can be stimulated by the hyperproliferation of tumor cells, increased cellular demand and environmental changes. Autophagy is caused by ULK1/2-Atg13-FIP200-Atg101 and the transmembrane autophagy protein Atg9A initiated, they were all recruited to the autophagosome formation site. Subsequently, they are recruited Beclin-1-Atg14-hp150-PIK3C3 to produce 1,2-palmitoylphosphatidylinositol-3-phosphate□PI3P□.PI3P absorbs downstream effectors WIPI1/2 and DFCP1, and binds to autophagy proteins, reorganizes and expands the isolation membrane to form autophagosomes[16, 17], Autophagosomes are the core of autophagy, a dual membrane structure, and the autophagosome is formed with the assistance of GABARAP, a member of the Atg8 family of autophagy-related proteins, which helps to maintain ULK1 activation and substrate phosphorylation during the final stage of autophagy formation until the ULK1 complex dissociates and the autophagosome closes[18], Interacts with NSF and TRPML3[19], and priority recruitment PLEKHM1 through the LIR module. Autophagy-lysosomal fusion driven by HOPS recruitment, HOPS is mediated by

GABARAP interaction with PLEKHM1[20] and eventually allow the encapsulated substrate to be degraded by lysosomes in the autophagic vesicles.

In recent years, autophagy has been found to be closely associated with the development of pancreatic cancer. Yang et al.[21]found that mouse glandular vesicle cells were more susceptible to pancreatic ductal Metaplasia and precancerous lesions after inhibiting autophagy. This demonstrates that autophagy has an anticancer effect in early tumorigenesis. Autophagy also plays a role in the development of pancreatic cancer. Not only is there an increased level of autophagy in pancreatic cancer tissues, but also an increased autophagy flux is found in the invaded nerve fibers and lymph nodes[22], And high levels of autophagy in the surrounding tissues of pancreatic cancer strongly suggest a poor prognosis for the patient [23]. This reflects the carcinogenic role of autophagy in the development of pancreatic cancer. However, when autophagy is overactivated, the degradation of large amounts of macromolecules, organelles, etc. can also lead to programmed cell death, which is also a way to inhibit pancreatic cancer cells. It has been shown that therapeutic measures such as cisplatin and ionizing radiation can activate autophagy and induce autophagic cell death in pancreatic cancer cells[24]. Triptolide has also been found to induce up-regulation of autophagy levels and autophagic death in pancreatic cancer cells[25].

Among the 42 patients with GABARAP-positive pancreatic cancer, single-factor survival analysis showed no significant difference between the clinicopathological factors and the overall survival of the chemotherapy group and the non-chemotherapy group ($P > 0.05$), while among the 34 patients with GABARAP-negative cancer, single-factor analysis showed that tumor site ($P=0.044$) and adjuvant chemotherapy ($P=0.005$) were significantly associated with overall survival. Since 76 patients underwent radical treatment for pancreatic cancer, and the prognosis of the GABARAP-positive group was significantly better than that of the negative group, we believe that chemotherapy drugs such as gemcitabine induced the autophagic death of pancreatic cancer cells that might be residual, which up-regulated the GABARAP level in the GABARAP-negative cancer cells and improved the prognosis. In pancreatic cancer cells with high GABARAP expression (i.e., pancreatic cancer tissues with high autophagy level), the autophagy induced by chemotherapy was not significant (could not further increase the autophagy level to induce autophagic death) or resisted, which was not significantly different from that without chemotherapy, suggesting that adjuvant chemotherapy may not be effective in these patients. For the first result, considering that none of the patients had undergone preoperative neoadjuvant chemotherapy and the autophagy level of normal pancreatic tissue was not high, the autophagy of pancreatic cancer cells was mainly caused by the harsh environment around the pancreatic cancer cells, i.e., there was insufficient blood supply, the probability of distant metastasis of pancreatic cells through blood vessels was low, and there were few residual tumor cells after radical resection, so the overall survival of patients was long. We believe that the high expression of GABARAP in patients whose tumors can be radically resected in the early stage reflects the good prognosis of the patients.

In our study, other clinicopathological factors such as sex, TNM stage, tumor classification, tumor size, lymph node metastasis, vascular infiltration, preoperative CA19-9 level, surgical modality and degree of

differentiation were not significantly associated with patient prognosis after radical pancreatic cancer treatment. However, this does not mean that these factors are not important to the prognosis of patients, it may be that the number of cases is too small or the follow-up time is insufficient, which needs further study.

Taken together, these findings suggest a possible complex relationship of GABARAP in pancreatic tumor progression and metastasis. In combination with the results of this study, we believe to some extent that GABARAP is overexpressed in pancreatic cancer and may inhibit pancreatic cancer progression through the autophagy pathway, thus providing a choice of therapeutic strategy, with the specific mechanism of action to be revealed by further studies.

Conclusions

1. GABARAP is highly expressed in pancreatic ductal adenocarcinoma tissue

2. In PDAC, GABARAP expression levels in pancreatic cancer tissues were significantly correlated with overall survival of patients, and the overall survival of patients with high expression was significantly higher than that of patients with low expression

3. In PDAC, GABARAP expression levels in pancreatic cancer tissues and postoperative adjuvant chemotherapy have independent value in assessing the prognosis of patients after radical pancreatic cancer treatment.

4. In PDAC, high GABARAP expression in pancreatic cancer tissues may be a predictor of poor adjuvant chemotherapy benefit in patients

Abbreviations

GABARAP:Gamma-Aminobutyric Acid Receptor-Associated Protein; ATP:Adenosine triphosphate; Unc-51:unc-51□Serine/threonine-protein kinase unc-51□,Uncoordinated protein 51; ULK1/2:Unc-51 Like Autophagy Activating Kinase 1/2; Atg:Autophagy-Related Protein; DMBA:Dimethylolbutanoic acid,[2,2-Bis(hydroxymethyl)butyric Acid]; FIP200:(Focal adhesion kinase,FAK),FAK-family interacting protein of 200 kDa; hp150:Chromatin assembly factor 1 subunit A□CAF-I p150□hp150; PIK3C3: Phosphatidylinositol 3-kinase catalytic subunit type 3 PI3P, Phosphatidylinositol 3-phosphate,1,2-dipalmitoyl; WIPI1/2:WD Repeat Domain, Phosphoinositide Interacting 1/2; DFCP1:Double FYVE-containing protein 1; NSF: N-ethylmaleimide-sensitive fusion protein/Vesicle-fusing ATPase; TRPML3: Transient receptor potential channel mucolipin 3/ Mucolipin-3; LIR: LC3 interacting region; LC3 Microtubule-associated proteins 1A/1B light chain 3; PLEKHM1: Pleckstrin Homology And RUN Domain Containing M member 1; HOPS: homotypic fusion and protein sorting; AJCC: American Joint Committee on Cancer; DAB: Diaminobenzidine; SPSS: Statistical Package for the Social Science; TNM: Tumor Node Metastasis; OR: Odds ratio; RPKM: Reads Per Kilobase of exon model per Million mapped reads; ALS1: Amyotrophic Lateral Sclerosis 1; GO: Gene Ontology; FYCO1: FYVE and coiled-coil domain-containing protein 1; JIP1:

C-Jun-amino-terminal kinase-interacting protein 1; TEX264: Testis Expressed 264; PI4K2A: Phosphatidylinositol 4-Kinase Type 2 Alpha; PtdIns4P: Phosphatidylinositol-4-phosphate; RAB7: (Rat sarcoma,Ras),Ras-related protein Rab-7a; siRNA: SmallinterferingRNA; GEM: Gemcitabine; VMP1: Vacuole Membrane Protein 1; 5-FU: 5-fluorouracil; CQ: Chloroquine

Declarations

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Author's contribution

ZC, TWH, TYY, YZR, WW, WZK and WWY conducted pathological design and analysis, ZC conducted sample collection and wrote the manuscript. XL and GY were stained and read by immunohistochemistry. All the authors read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Ethics approval and consent to participate

All tissue samples were obtained with patients writing consent and the study was approved by the ethical committee of Huadong Hospital Affiliated to Fudan University (NO.2018K006) and performed in accordance with the ethical guidelines of the Declaration of Helsinki.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1 Relationship between GABARPAP expression and clinicopathological parameters in pancreatic cancer tissue

Variable	No. of cases	GABARAP-positive cases□%□	Univariate *P*
Sex			
Females	26	13(50.0%)	0.506
Males	50	29(58.0%□	
Age(years)			
□65	34	19(55.9%)	0.922
≥65	42	23(54.8%)	
Tumor location			
Head	48	24(50.0%)	0.227
Corpus or Tail.	28	18(64.3%)	
TNM			
□	6	3(50.0%)	0.925*
□	57	31(54.4%)	
□	13	8(61.5%)	
Tumor size			
□3cm	18	10(55.6%)	0.977
≥3cm	58	32(55.2%)	
Vascular invasion			
No	57	33(57.9%)	0.424
Yes	19	9(47.4%)	
Neuroaggression			
No	20	9(45.0%)	0.282
Yes	56	33(58.9%)	
Lymph node metastasis			
No	35	18(51.4%)	0.534
Yes	41	24(58.5%)	
Degree of differentiation			
undifferentiated	12	9(75.0%)	0.162*
neutralization	63	33(52.4%)	
highly differentiated	1	0(0.00%)	
Preoperative level of CA19-9			
Normal	8	5□62.6%□	0.663
Elevate	68	37□54.4%□	
Post-operative chemotherapy			
No	16	9□56.3%□	0.929
Yes	60	33□55.0%□	

*Fisher text

Table 2 Expression of GABARAP in pancreatic adenocarcinoma tissues and normal pancreatic tissues at the paracellular level

Group	No. of cases	No. of GABARAP positive（%）	No. of GABARAP negative（%）
Pancreatic cancer tissue	76	42（55.26%）	34（44.74%）
Paraneoplastic normal pancreatic tissue	76	12（21.42%）	64（78.58%）

Cardinality test χ=25.850,P0.001

Table 3 One-factor survival analysis of survival after radical pancreatic cancer treatment

Variable	No. of cases	Median DFS (months)	Univariate *P*	Median OS (months)	Univariate *P*
Sex					
Females	26	10.8	0.913	18.9	0.683
Males	50	12.9		21.3	
Age (years)					
65	34	15.4	0.241	24.6	0.048
≥65	42	11.4		17.4	
Tumor location					
Head	48	12.6	0.251	18.6	0.018a
Corpus or Tail	28	13.2		22.7	
TNM					
	6	10.5	0.978	17.4	0.611
	57	12.8		18.4	
	13	16.3		22.8	
Tumor size					
3cm	18	12.1	0.543	18.1	0.509
≥3cm	58	12.8		19.8	
Vascular invasion					
No	57	12.9	0.356	22.6	0.505
Yes	19	11.4		18.4	
Neuroaggression					
No	20	12.4	0.140	23.7	0.597
Yes	56	12.9		19.1	
Lymph node invasion					
No	35	13.8	0.189	22.6	0.594
Yes	41	11.0		19.0	
Degree of differentiation					
undifferentiated	12	13.9	0.369	25.1	0.478
neutralization	63	12.7		18.9	
highly differentiated	1	33.3		33.3	
Preoperative level of CA19-9					
normal	8	16.6	0.750	19.2	0.912
elevate	68	12.6		19.7	
Post-operative chemotherapy					
No	16	12.2	0.524	15.6	0.018
Yes	60	12.8		22.5	
GABARAP (cancerous tissue)					

negative					
positive	34	11.8	0.701	17.4	0.015b
	42	12.9		22.0	

a：Survival curves cross, overall survival period：8.8 month, Log-rank test P-value

b：Survival curves cross, overall survival period：8.8 month, Log-rank test P-value

Table 4 Cox regression multifactorial analysis of risk factors for overall survival after radical pancreatic cancer treatment

Factors	OR-value	Exp(B)	P-value
Age（≥65y）	1.697	0.529	0.216
Tumor site（body and tail of pancreas）	0.458	-0.781	0.125
Postoperative chemotherapy（Yes）	0.413	-0.885	0.038
GABARAP（positive）	0.440	-0.821	0.044

Table 5 Cox regression multifactorial analysis of risk factors for overall survival after radical pancreatic cancer treatment in 34 patients with GABARAP-negative cancer tissue

Factors	OR-value	Exp(B)	P-value
Tumor site（body and tail of pancreas）	0.317	-1.150	0.137
Postoperative chemotherapy（Yes）	0.340	-1.078	0.041

Figures

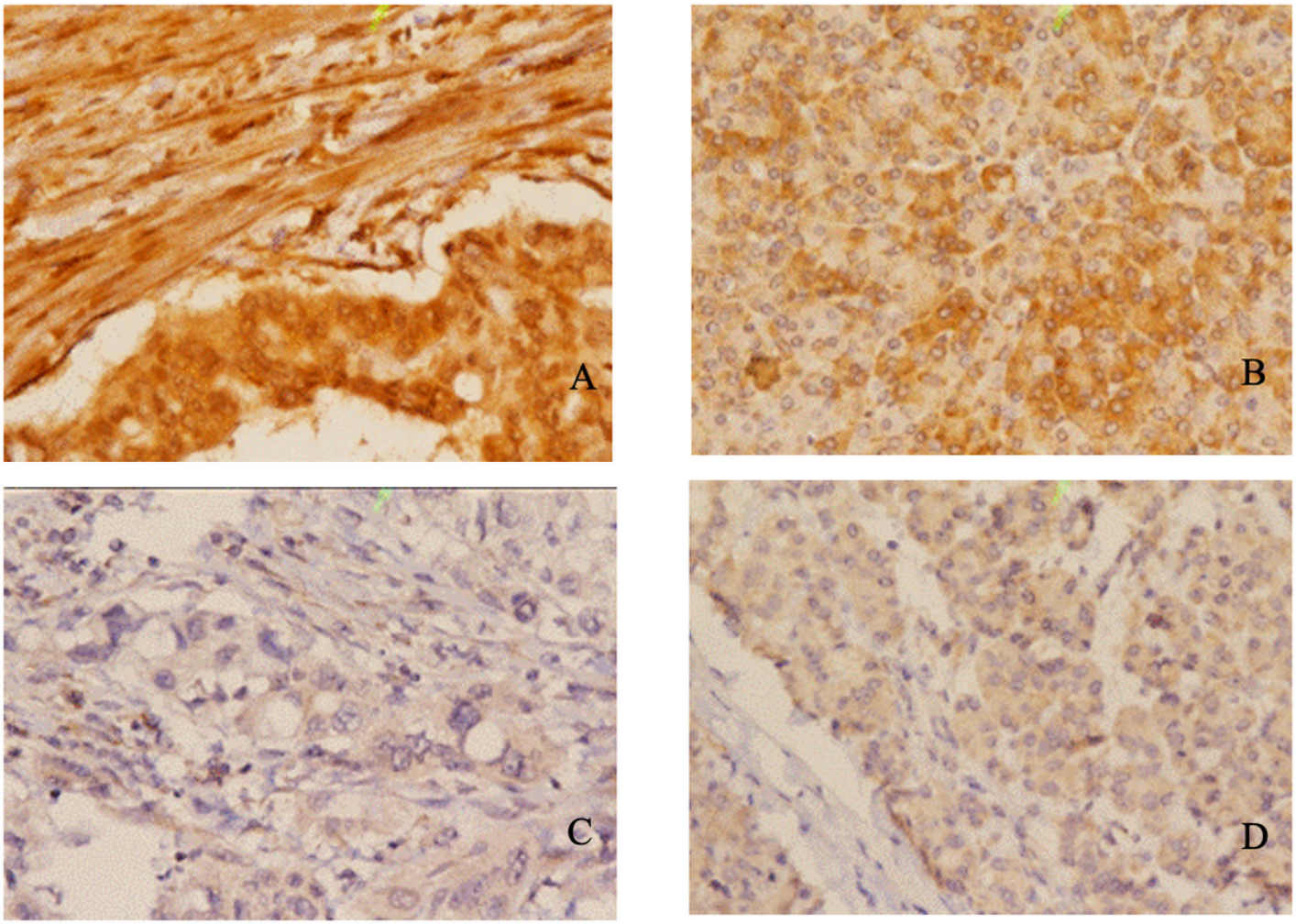

Figure 1

A: Immunohistochemically positive control staining for GABARAP in human pancreatic adenocarcinoma tissue（400 magnification） B: Immunohistochemically positive results for GABARAP in paraneoplastic normal pancreatic tissue （400 magnification）C: Immunohistochemically negative control staining for GABARAP in human pancreatic adenocarcinoma tissue （400 magnification）D: Immunohistochemically negative results for GABARAP in paraneoplastic normal pancreatic tissue（400 magnification）

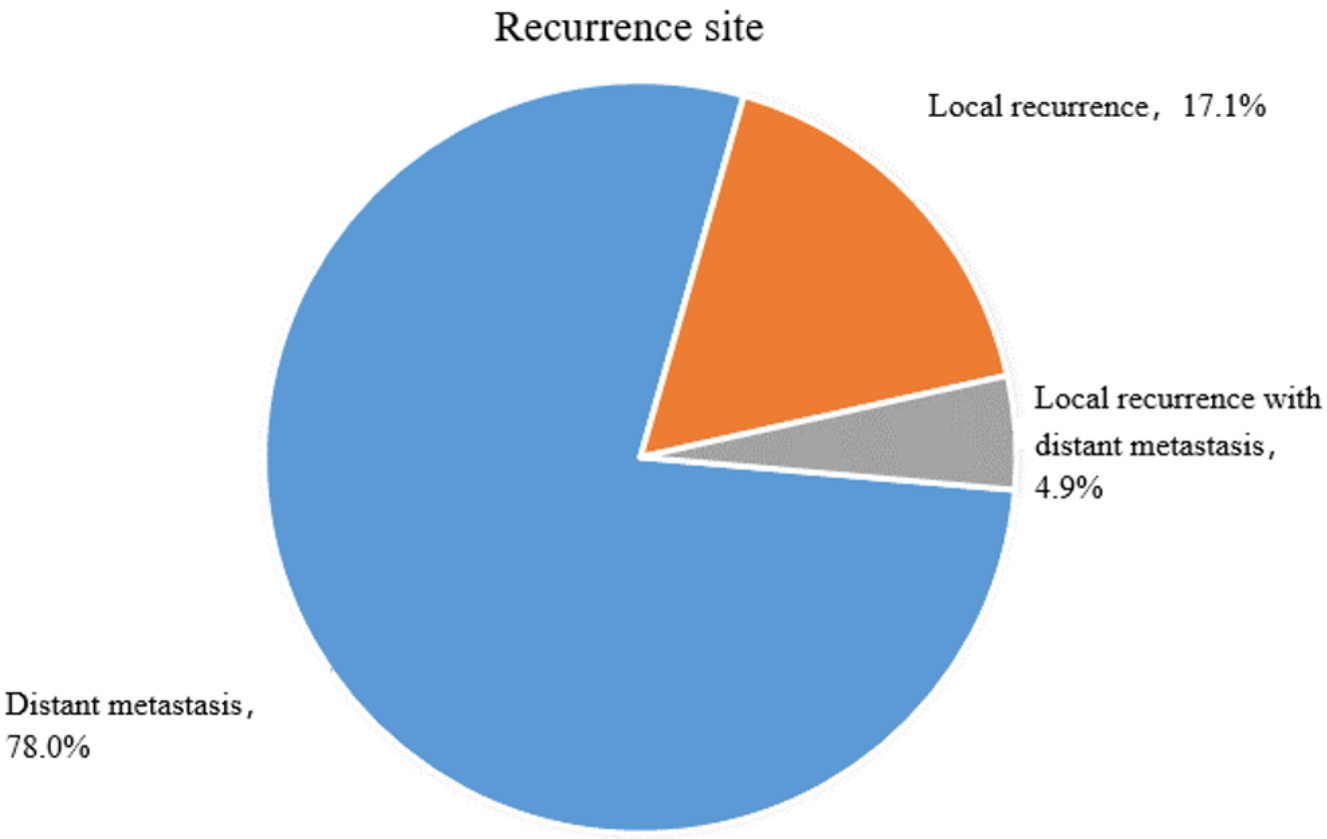

Figure 2

Recurrence site

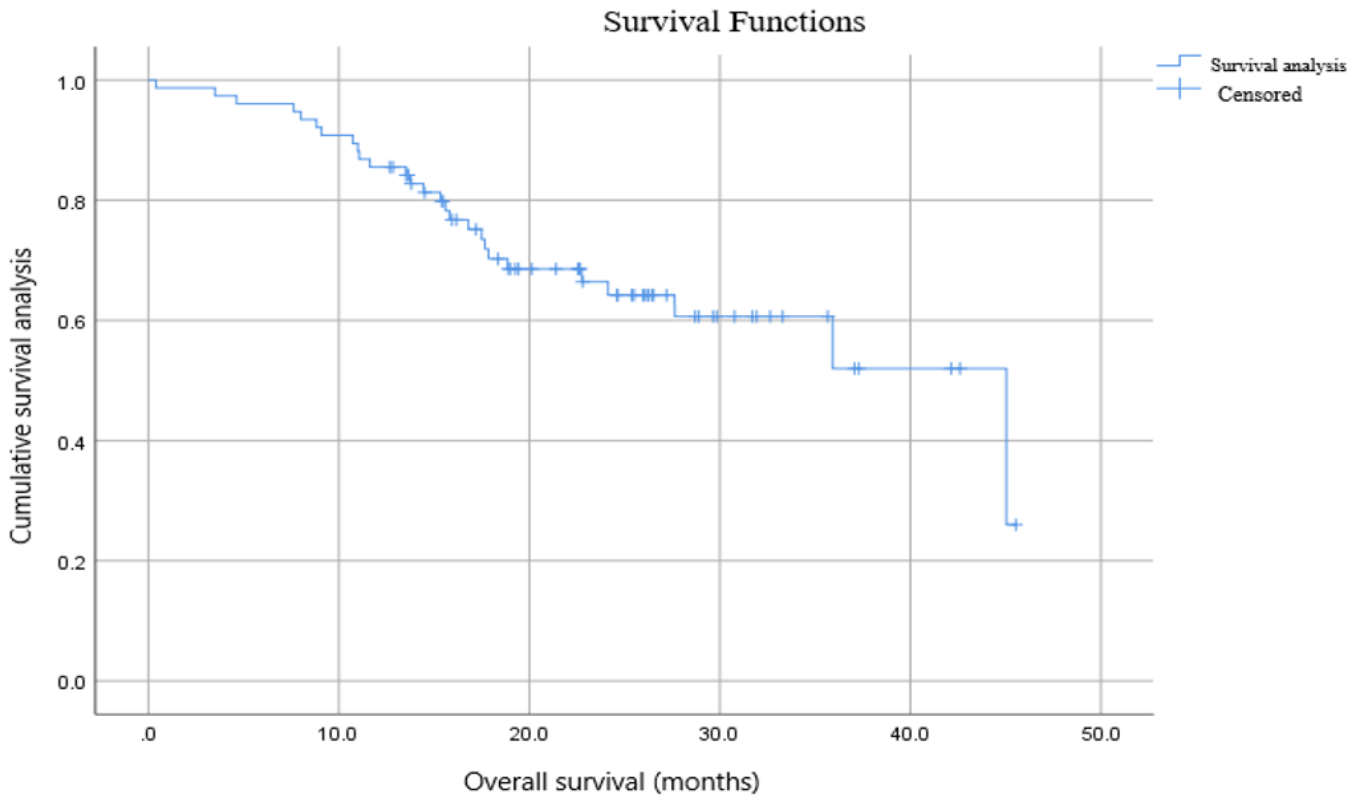

Figure 3

Overall survival of 76 patients

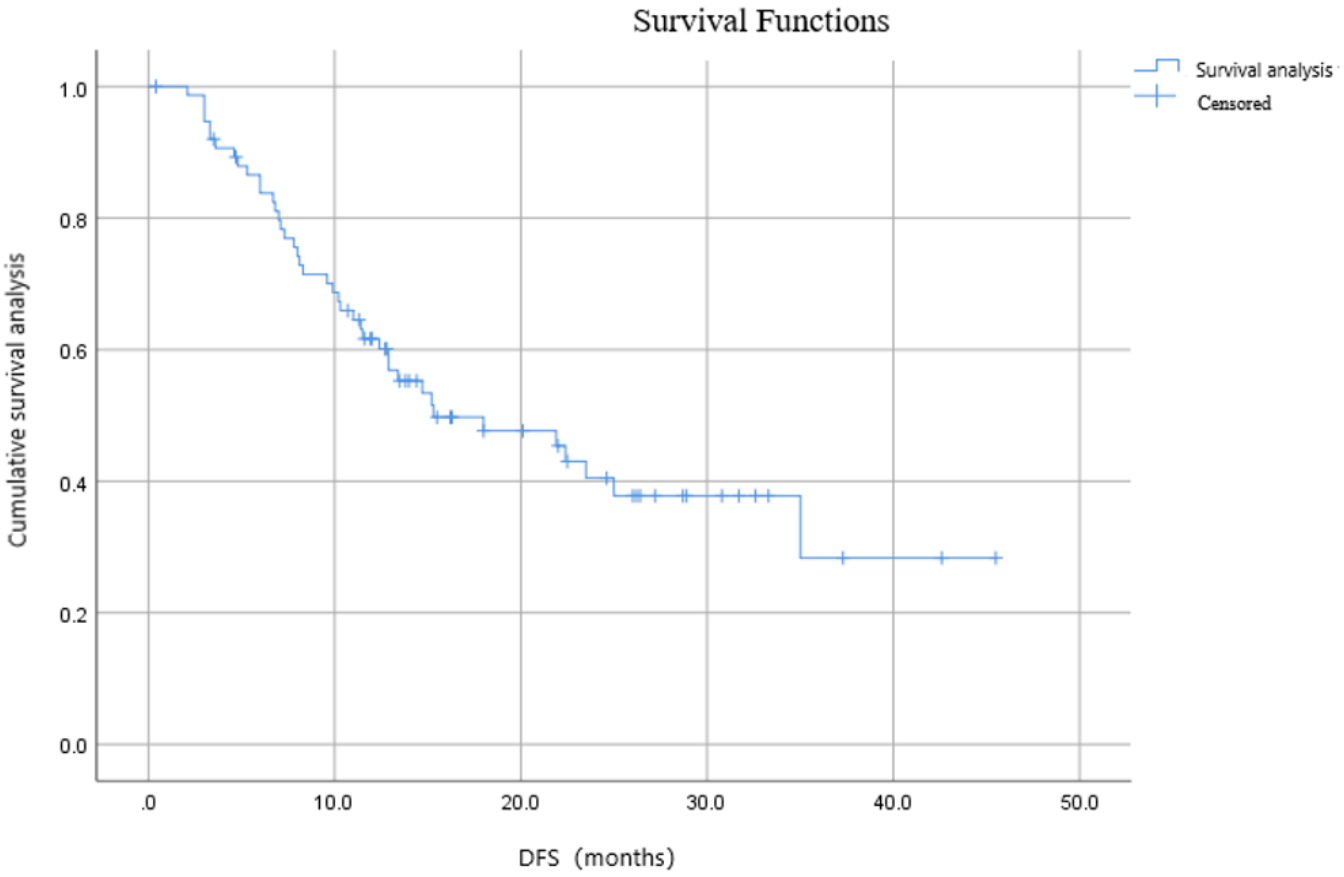

Figure 4

Disease-free survival of 76 patients

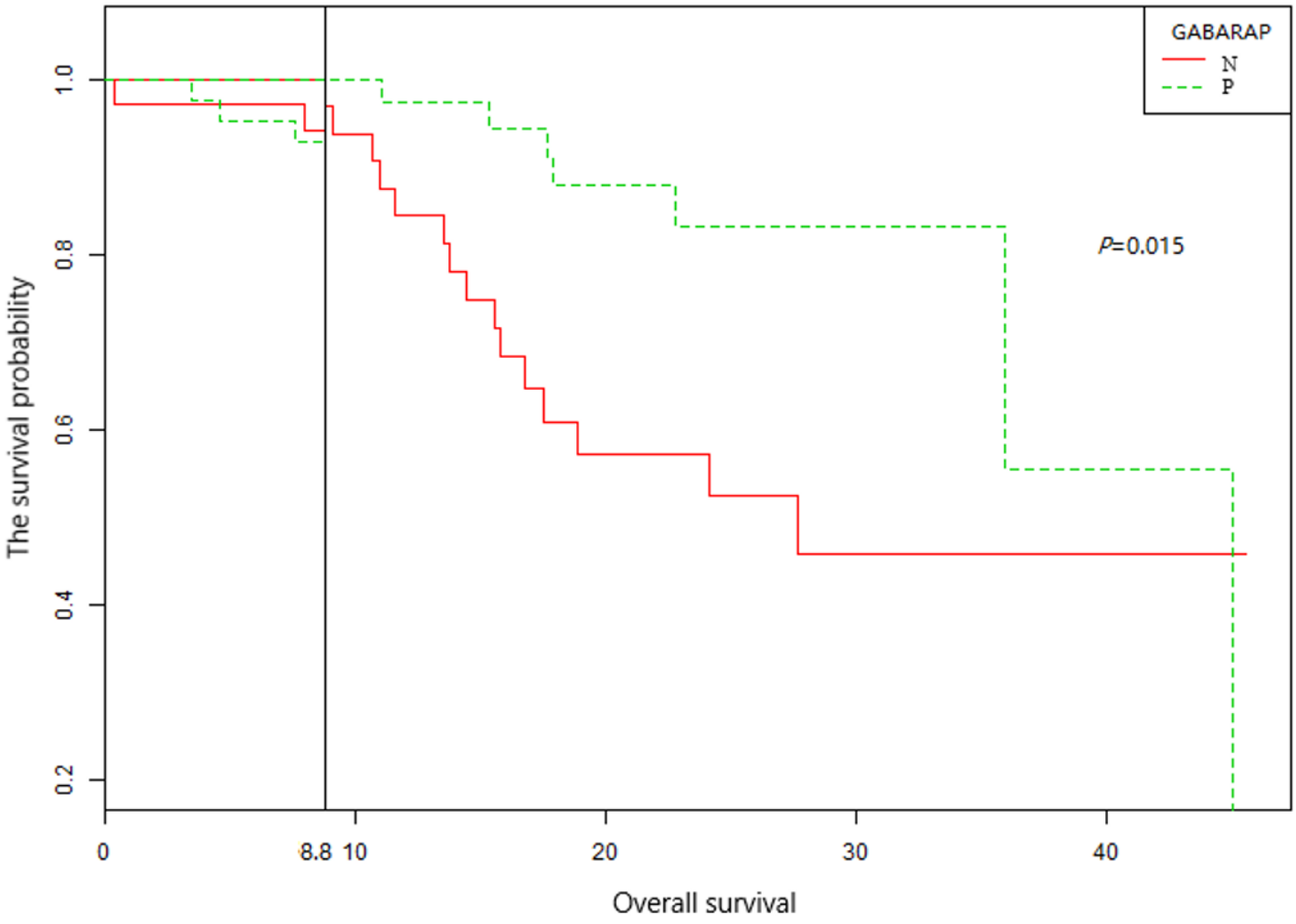

Figure 5

Relationship between GABARAP and overall survival in cancer tissue（N：negative；P:positive）

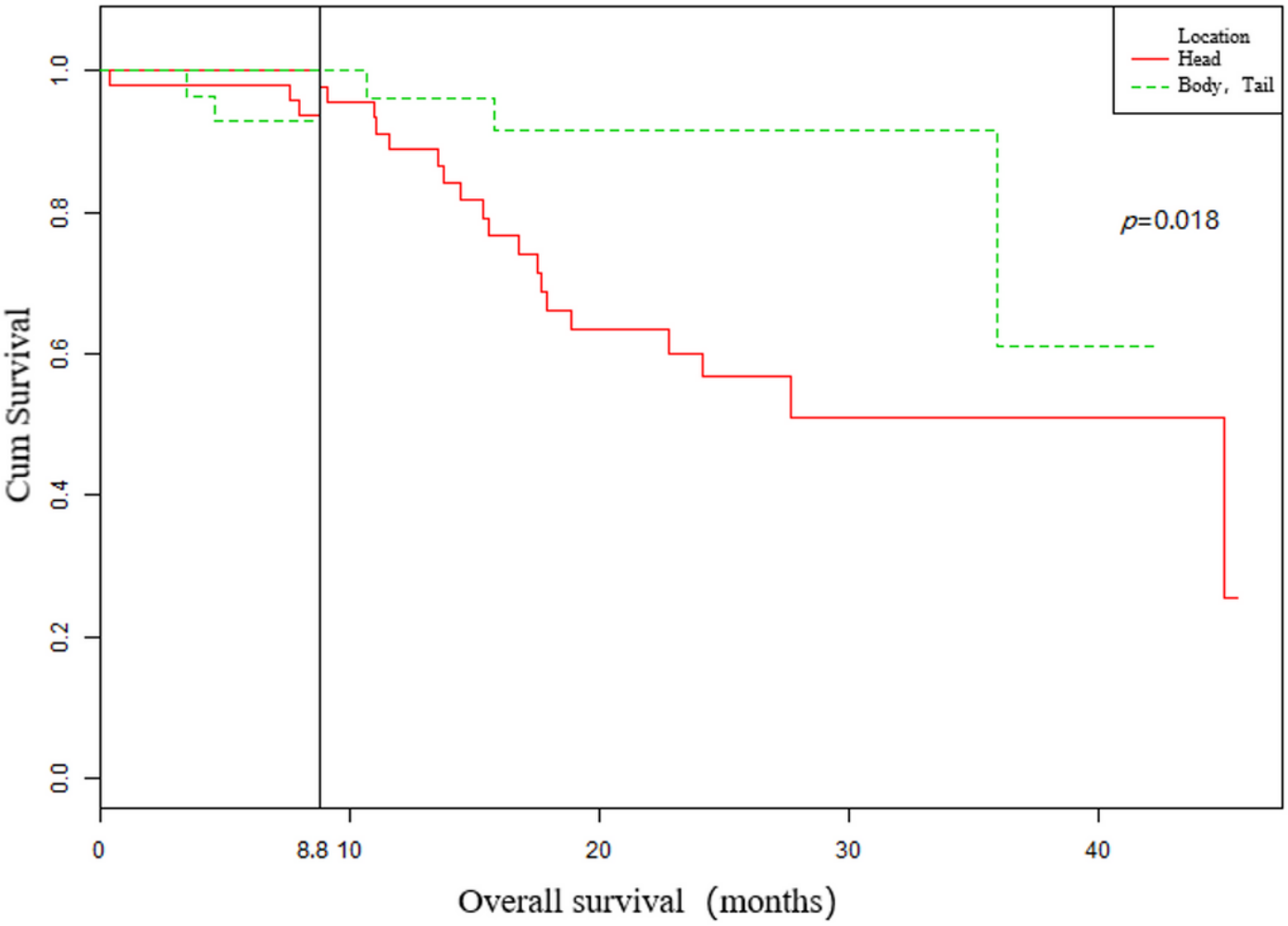

Figure 6

Tumor site versus overall patient survival

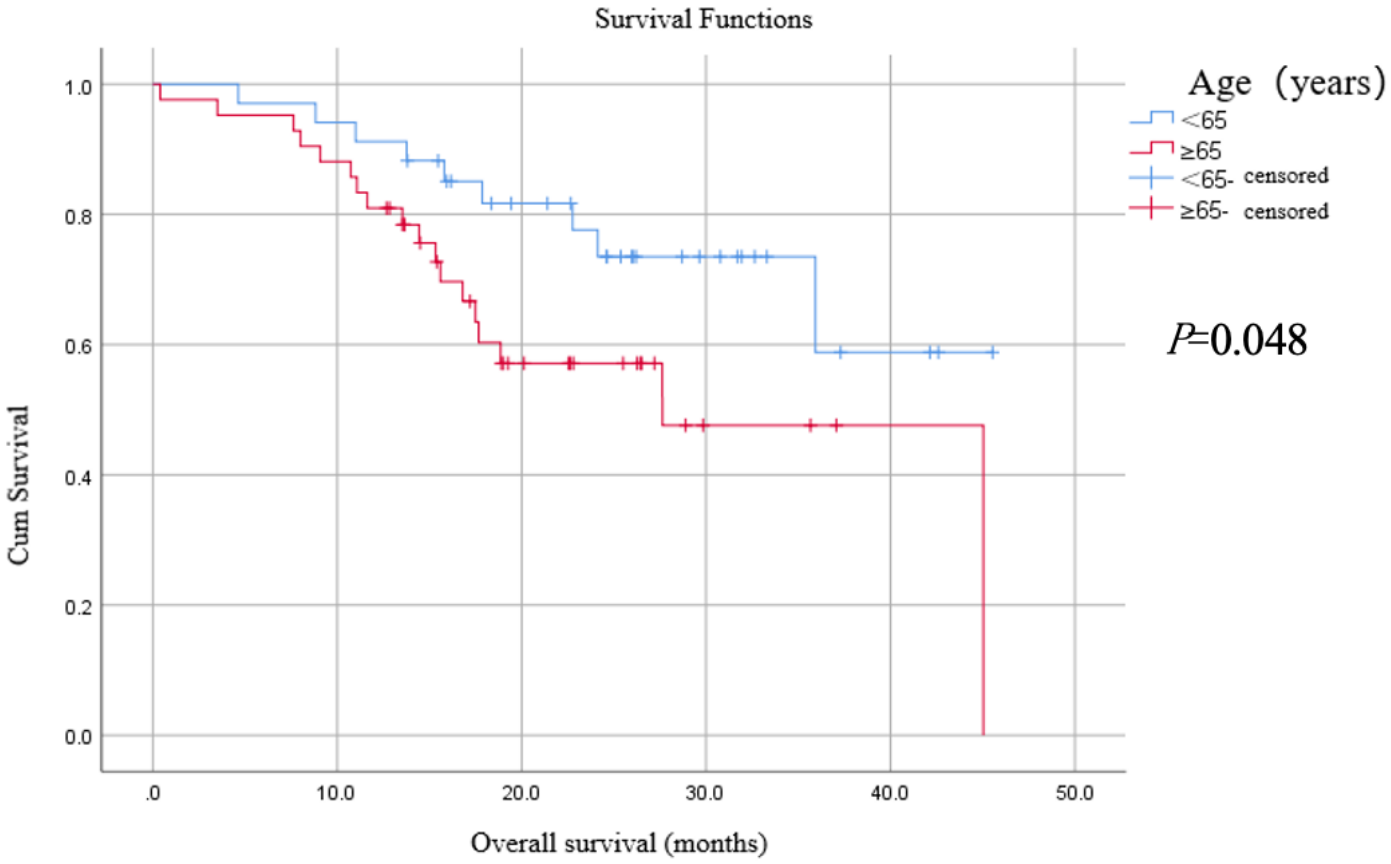

Figure 7

Relationship between age and overall patient survival

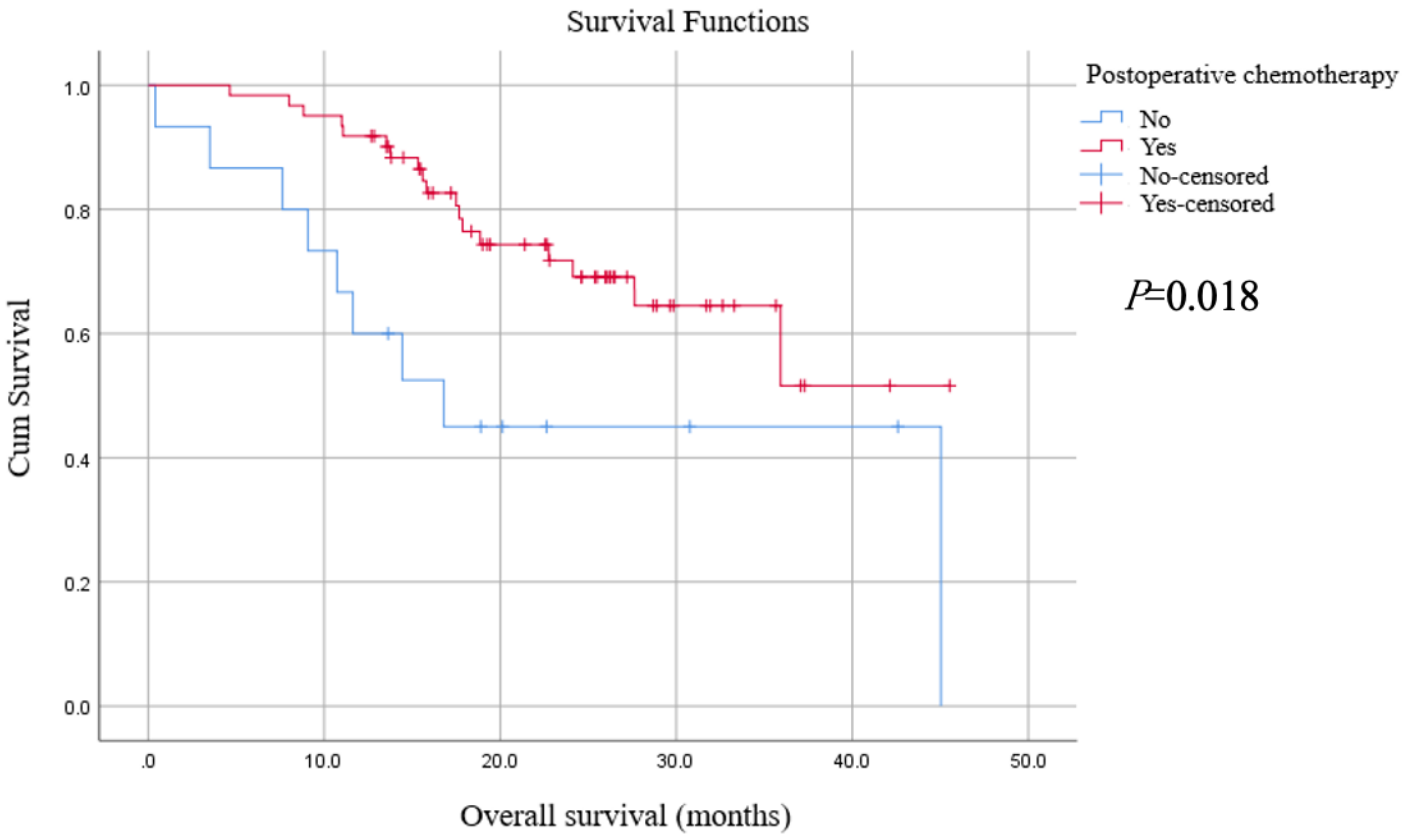

Figure 8

Relationship between postoperative adjuvant chemotherapy and overall patient survival

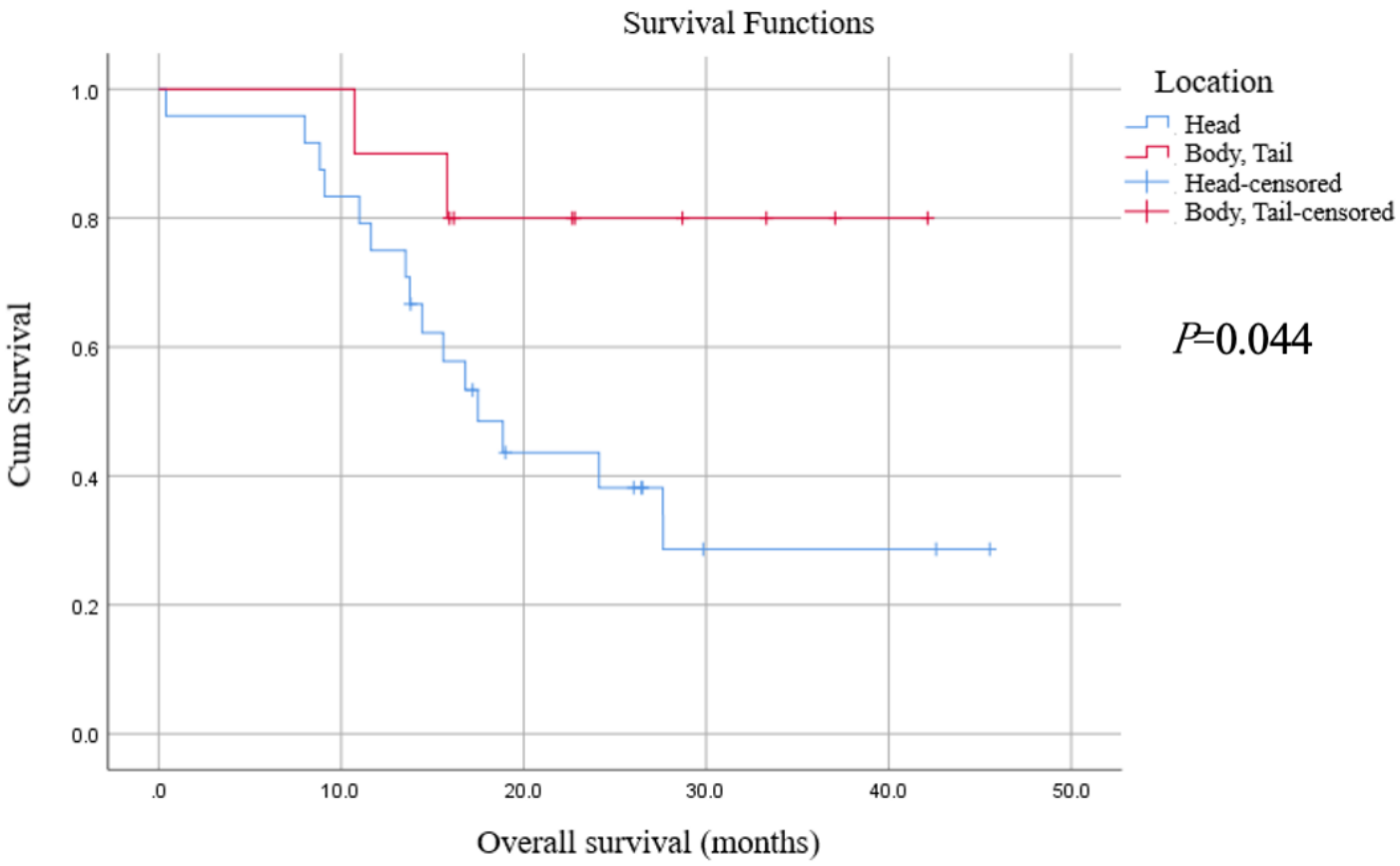

Figure 9

Comparison of pancreatic head and pancreatic tail survival analysis of 34 patients with GABARAP-negative cancer tissue

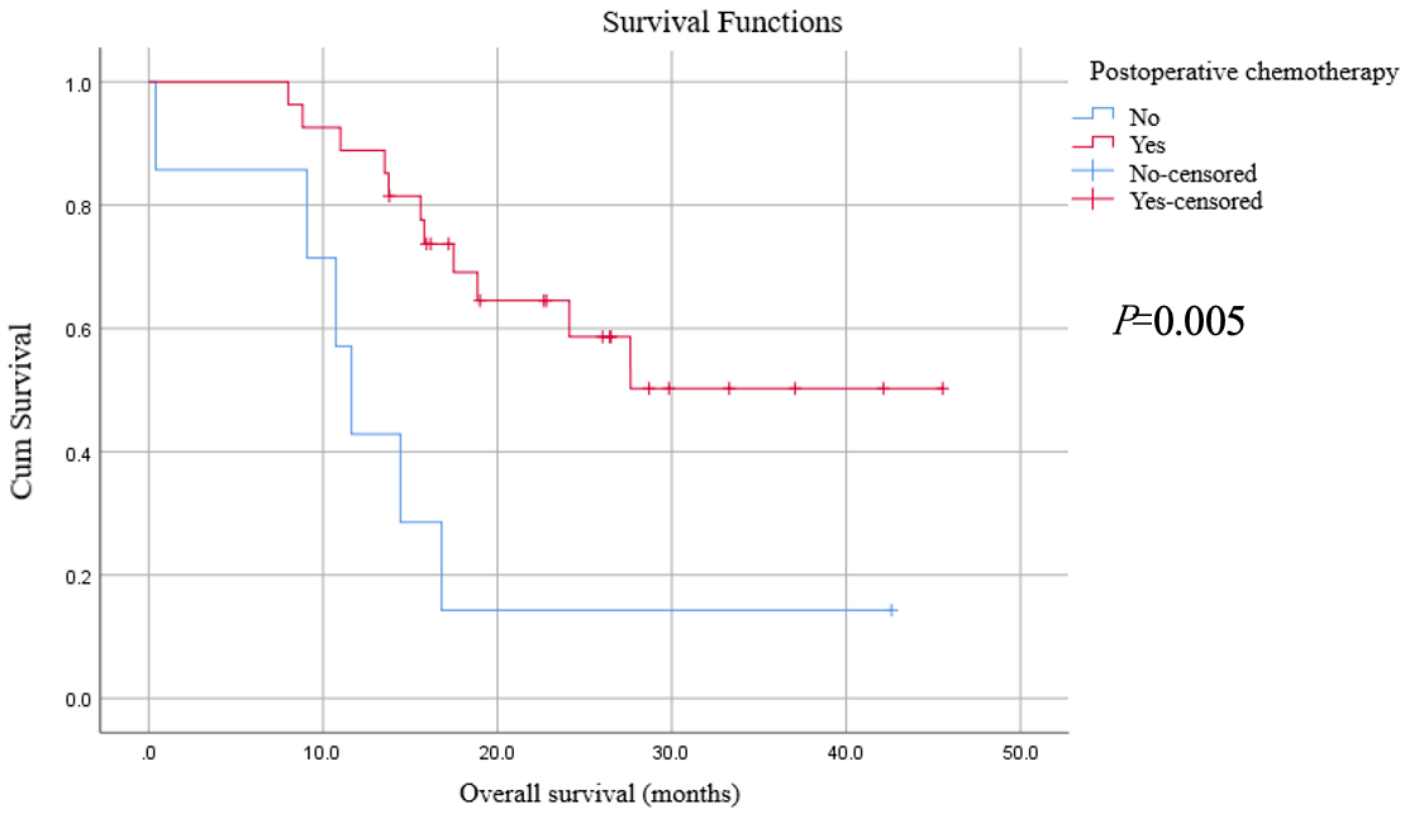

Figure 10

Comparison of chemotherapy and non-chemotherapy survival analysis of 34 cancer tissues with GABARAP-negative group

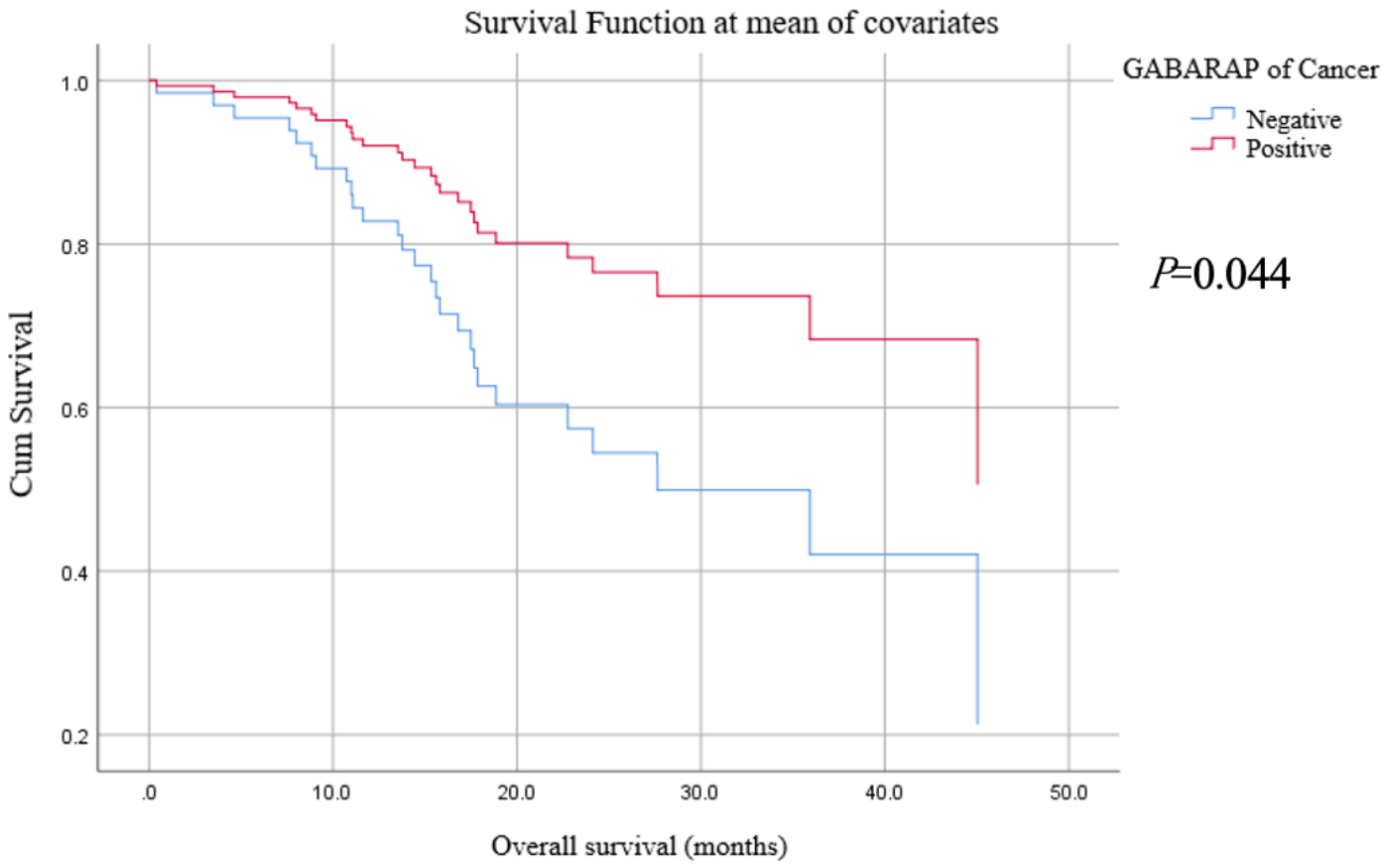

Figure 11

Cox regression multifactorial analysis of GABARAP in relation to total survival

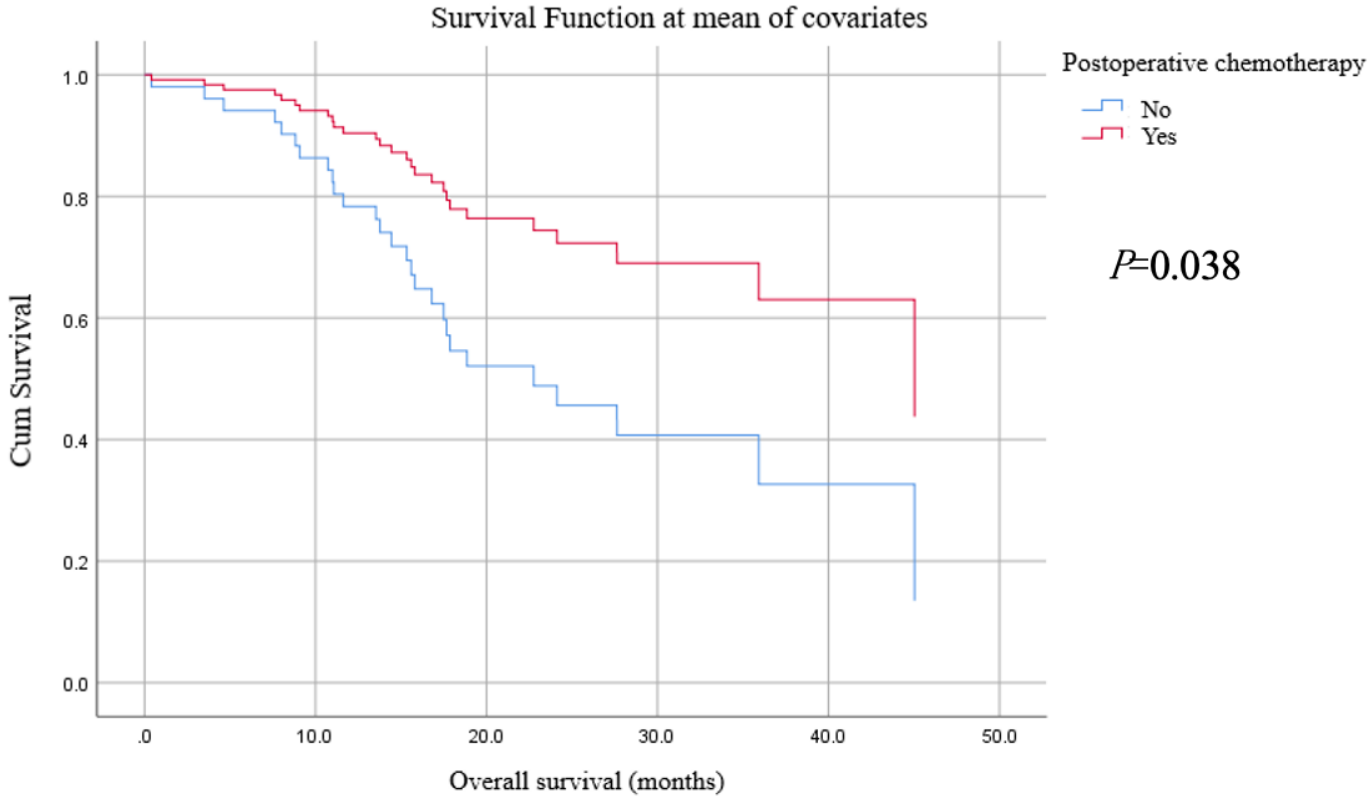

Figure 12

Cox regression multifactorial analysis of the relationship between adjuvant chemotherapy and overall survival

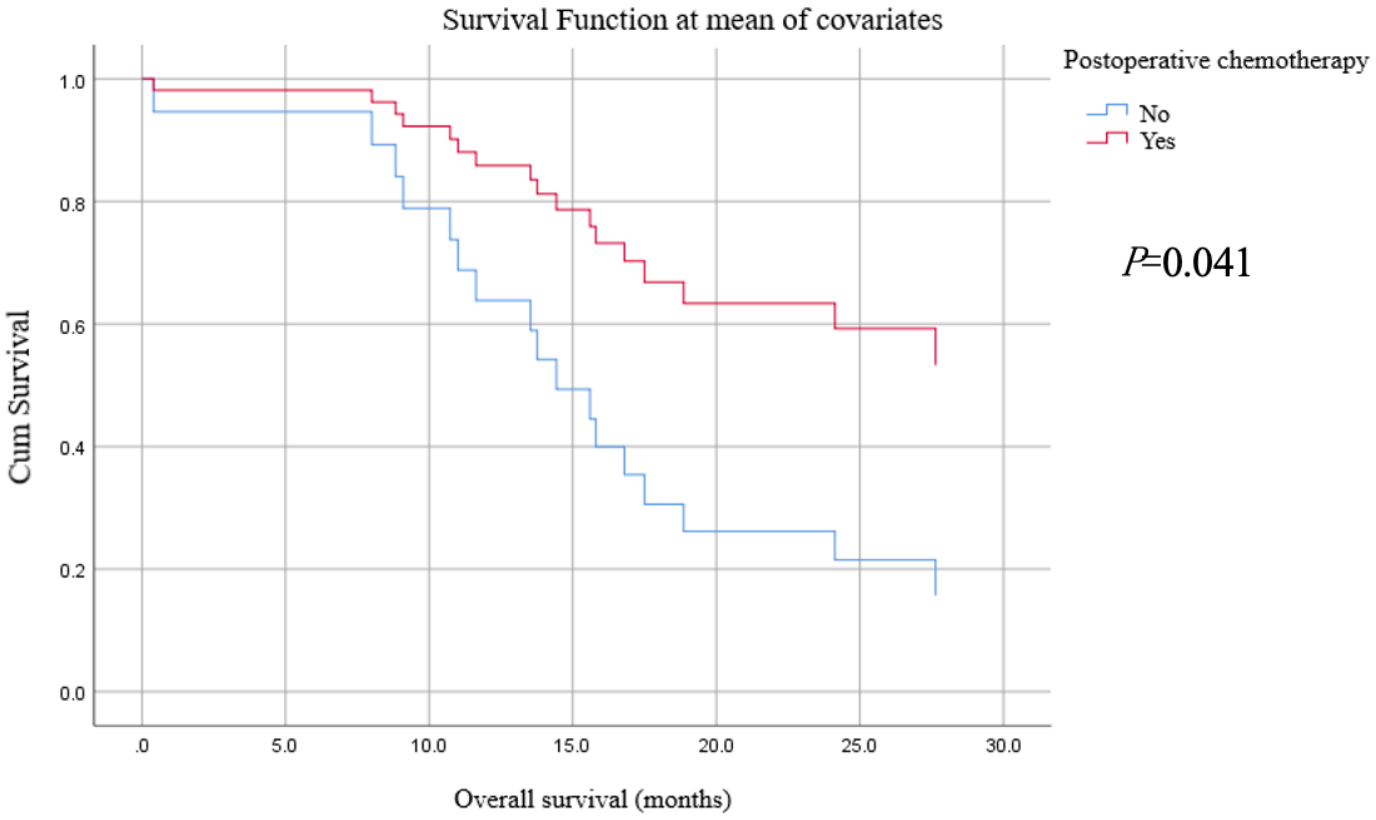

Figure 13

Cox regression multifactorial analysis of the relationship between GABARAP-negative adjuvant chemotherapy and overall survival in 34 cases of cancer tissue