

CASE REPORT

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## Poorly cohesive adenocarcinoma of the ampulla of Vater: a case report

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### Abstract

A 47-year-old Japanese male was submitted to pancreaticoduodenectomy for an ampullary cancer. Pathologically, the ampullary cancer was poorly cohesive adenocarcinoma without tubular structure. Moreover, locoregional lymph nodes were swollen with hypervascularity, plasmacytes infiltration, and hemorrhage. Our case seems to be different from usual poorly differentiated adenocarcinoma.

**Keywords:** Ampullary cancer, Cohesive adenocarcinoma, Poorly differentiated adenocarcinoma

### Background

Ampullary carcinoma is a malignant tumor arising in the last centimeter of the common bile duct, and patients with these tumors have been reported to have a relatively favorable prognosis after surgical resection [1]. Among the tumors originated from the ampulla of Vater, poorly differentiated adenocarcinoma is a very rare disease [2] and the clinical outcomes of the patients are undetermined. On the other hand, Castleman's disease (CD) is a lymphoproliferative disorder which was first described by Dr. Benjamin Castleman in 1956 and which often develops in the retroperitoneal lymph nodes [3].

Here, we present a rare case of poorly cohesive adenocarcinoma of the ampulla of Vater without tubular structure, the histology of which is similar to poorly cohesive adenocarcinoma of the stomach. The swollen locoregional lymph nodes showed marked plasma cells infiltration, vascular proliferation like CD and hemorrhage, but no lymph node metastasis of the tumor was found.

### Case presentation

The patient was a 47-year-old man who had no pathological antecedents. He admitted to our hospital with hyperbilirubinemia of 1.6 mg/dl at health screening, with appetite loss and epigastralgia appearing 6 months ago. Carbohydrate antigen 19-9 and carcinoembryonic antigen

were normal. Abdominal computed tomography and magnetic resonance imaging showed a dilatation of the common bile duct and a stone in the gallbladder. Neither tumor nor lymph nodes swelling were observed. No stones or abnormal arrangement of the pancreaticobiliary ductal union were found by an endoscopic retrograde cholangiopancreatography. Gastrointestinal endoscopy showed irregularly shaped concavity on the ampulla of Vater, and histology of the biopsy revealed signet ring cell carcinoma.

A pancreaticoduodenectomy was performed. The final pathological diagnosis was poorly cohesive adenocarcinoma including signet ring cell carcinoma component in the Vater's ampulla without lymph node metastases (Fig. 1). The carcinoma consisted of complete poorly differentiated carcinoma cells most of which showed non-cohesive pattern, and tubular structure was not seen in any sections examined for pathological analysis (Fig. 2). Tumor invasion was seen around the common duct, bile duct, and pancreatic duct in the ampulla of Vater and the duodenum wall. A little lymphatic and vascular invasion was observed. Perineural invasion was little seen.

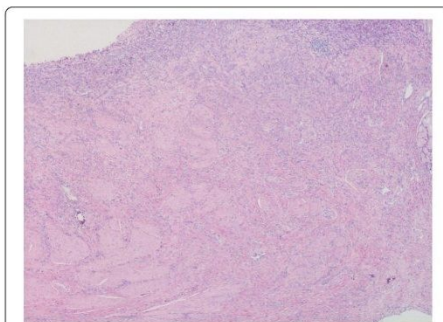
The locoregional lymph nodes were swollen with hemorrhage in the capsule. Lymph follicles were atrophic, and vascular hyperplasia was found in the germinal centers (Fig. 3). Marked plasma cell infiltration was seen in the interfollicular stroma (Fig. 4). However, hyalinization of the vascular wall was not seen. Histological findings resemble those of CD but are slightly different. Immunohistochemically, plasma cells in the interfollicular

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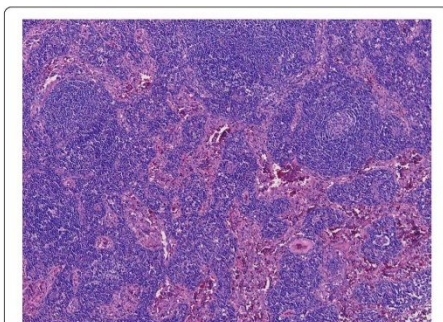
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**Fig. 1** Infiltrating carcinoma cells shows scirrhous appearance in the ampulla of Vater



**Fig. 3** Lymph follicles were atrophic, and vascular hyperplasia was found in the germinal centers

region were evenly positive for kappa and lambda light chain, which meant no evidence of clonal proliferation.

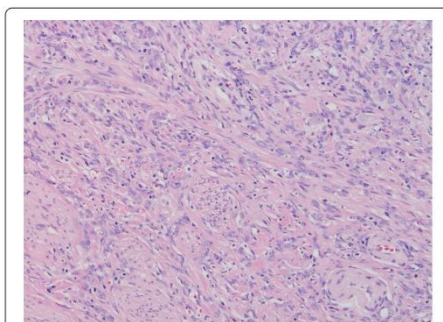
According to the TNM classification, the tumor was classified as pT3 N0 M0 ly1 v1 pn0 pstageII. The patient remained well and had no evidence of locoregional and metastatic recurrence during the 28 months of follow-up.

#### Discussion

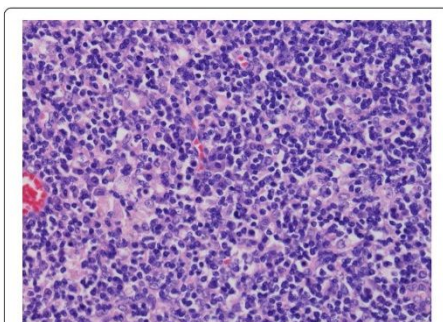
The primary adenocarcinoma of the ampulla of Vater is a rare tumor, and most ampullary adenocarcinomas are well differentiated [2]. Ampullary carcinomas are thought to arise from the glandular epithelium of the ampulla of Vater [4]. It has been suggested that molecular biologically ampullary carcinoma is different from bile duct and pancreatic carcinomas, whereas that share the same molecular biological characteristics with duodenal carcinomas [1]. The clinical outcome of ampullary carcinoma has been reported to be better than those of bile duct and

pancreatic carcinomas [1]. Thus, it would be important to distinguish the origin of carcinomas developed around the region of the ampulla of Vater.

The present case was diagnosed as ampullary carcinoma, since the tumor invaded both the common bile duct and the biliary and pancreatic ducts in the duodenal wall. However, the tumor in our case was non-cohesive type of poorly differentiated adenocarcinoma, in which glandular structure was not observed in any of specimens, and the histology resembled scirrhous carcinoma of the stomach, which was classified into poorly cohesive carcinoma according to World Health Organization (WHO) classification. To our knowledge, this type of carcinoma is uncommon in any of the ampulla of Vater, pancreatic duct, and bile duct [2, 5]. Thus, poorly cohesive carcinoma was listed only in the stomach, but not in the bile duct, pancreas, and the ampullary region in WHO classification.



**Fig. 2** The proliferating tumor cells consist of poorly cohesive adenocarcinoma without tubular structure



**Fig. 4** Marked plasma cell infiltration was observed in the interfollicular stroma

On the other hand, tumors of ampulla of Vater are categorized into bile duct carcinoma in Japanese classification [6]. Most extrahepatic cholangiocarcinomas have been reported to be histologically well-to-moderately differentiated adenocarcinomas [7]. As far as we researched in the PubMed, there has been no report about exclusively poorly cohesive adenocarcinoma of periampullary region. Thus, our case which consists of pure poorly cohesive carcinoma cells without tubular structure is thought to be quite rare.

However, our case was classified into poorly differentiated type of tubular adenocarcinoma (tub3) according to the General Rules for Surgical and Pathological Studies on Cancer of the Biliary Tract, Japanese Society of Biliary Surgery [6], because Japanese classification also did not have poorly cohesive carcinoma in the bile duct carcinoma as same as WHO classification.

Our patient underwent curative pancreatoduodenectomy. The poorly differentiated adenocarcinoma has been reported to exhibit higher probability of recurrence and show poor prognosis compared with other histological types after the curative resection [8–11]. The prognostic factors of ampullary carcinoma after resection include lymph node metastasis, pancreatic invasion, and perineural invasion [12, 13]. These histological findings are also the most important prognostic factors for bile duct carcinoma after curative resection [14, 15]. However, our case showed a little vascular invasion and perineural invasion without nodal metastasis in spite of the case of poorly differentiated carcinoma, which might indicate better prognosis than usual poorly differentiated adenocarcinoma.

Histopathologically, the cause of lymph node swelling was not due to the tumor metastasis but the CD-like change and hemorrhage. CD is a rare atypical lymphoproliferative disorder. And soon after the original presentation of Castleman et al., CD has been subdivided into a hyaline vascular and plasma cell histopathological pattern, with intermediate variants [16]. The pathological findings of the locoregional lymph nodes in our case revealed hypervascular germinal centers with marked interfollicular plasma cell infiltration, which are common features of CD. However, hyalinization of the proliferating vessel was not observed, which was different from CD. The patient has no past history related to immune dysregulation. Therefore, we could not definitely diagnose the patient as CD.

### Conclusions

We report a rare case of poorly cohesive adenocarcinoma in the ampulla of Vater with enlarged lymph nodes like CD. The relation between clinicopathological features and the long-term outcomes are not clear only in this case. The histopathological findings of our case

seem to be different from those of poorly differentiated adenocarcinoma, and it would be better to add poorly cohesive adenocarcinoma to the histopathological classification of the ampulla of Vater and bile duct.

### Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

### Abbreviations

CD: Castleman's disease; WHO: World Health Organization.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

HT and SS participated in the patient's care, research design, performance of the research, data analysis, and writing of the paper. KH, TY, YT, TF, SK, and MF participated in the patient's care and data collection. HN and HK participated in revising the manuscript critically. All authors read and approved the final manuscript.

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## Original Article

# Prognostic significance of $\beta$ 2-adrenergic receptor expression in non-small cell lung cancer

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**Abstract:** Background: The  $\beta$ 2-Adrenergic receptor ( $\beta$ 2-AR) is associated with tumor growth and progression. However, the clinical significance of  $\beta$ 2-AR expression in patients with non-small cell lung cancer (NSCLC) remains unclear. Methods: Three hundred twenty-eight patients with surgically resected NSCLC were retrospectively investigated. Tumor sections were stained by immunohistochemistry for assessing  $\beta$ 2-AR and Ki-67 expression and microvessel density (MVD), which was using CD34 levels. Results:  $\beta$ 2-AR was positively expressed in 27% of all patients, in 29% of adenocarcinoma (AC) patients, and in 24% of non-AC patients. In AC patients,  $\beta$ 2-AR expression was significantly correlated with lymphatic permeation ( $r=0.240$ ;  $P<0.001$ ), vascular invasion ( $r=0.239$ ;  $P<0.001$ ), and Ki-67 expression ( $r=0.175$ ;  $P=0.009$ ). However, this correlation was not observed in non-AC patients. Positive  $\beta$ 2-AR expression was identified as a negative predictor for worse outcomes in AC patients, particularly in those with stage I tumors. Multivariate analysis confirmed that  $\beta$ 2-AR expression was an independent factor for predicting poor progression-free survival in stage I AC patients (HR=2.220; 95% CI, 1.077-4.573;  $P=0.031$ ). Conclusion:  $\beta$ 2-AR expression is an independent prognostic factor for early-stage AC patients.

**Keywords:** Lung cancer, adenocarcinoma, stage I,  $\beta$ 2-adrenergic receptor, prognostic factor, immunohistochemistry

## Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. Despite years of research, the prognosis for patients with lung cancers remains dismal, with a 5-year survival rate of 14% [1]. In addition, patients with stage I non-small cell lung cancer (NSCLC) have a risk for disease recurrence even if the tumor is completely resected via surgery [2]. Thus, assessing the potential of established biomarkers for predicting the outcome and response to specific anti-cancer therapies is vital for improving the prognosis in NSCLC patients. Tumor staging and performance status are currently the most powerful prognostic predictors in patients with NSCLC [3]. Recent large-scale studies have demonstrated that sex, smoking history, and histology may also affect the treatment outcome in patients with NSCLCs [4-6].

The  $\beta$ 2-adrenergic receptor ( $\beta$ 2-AR), a member of the transmembrane G protein-coupled receptors (GPCRs) family, initiates multiple signaling cascades and regulates cell proliferation through a classical cyclic-adenosine-monophosphate (cAMP)/protein kinase A (PKA) pathway [7, 8]. Tumor recurrence is a multifactorial process that includes tumor cell migration, invasion, and metastasis. Neurotransmitters and chemokines are ligands that bind to GPCRs and act as prominent regulatory factors in tumor recurrence [9, 10]. It has recently been reported that  $\beta$ -adrenergic activation of the cAMP-PKA signaling pathway via  $\beta$ 2-AR could affect angiogenesis followed by cell growth and proliferation in ovarian cancer cells [11].

$\beta$ 2-AR overexpression has been reported in various human cancers, including breast cancer, oral cancer, prostate cancer, melanoma,

## $\beta$ 2-AR expression in NSCLC

and hepatocellular carcinoma, and it can lead to poor clinicopathological features such as tumor recurrence, metastasis, and reduced survival [12-16]. The results of these studies suggest that  $\beta$ 2-AR may be a cancer-relevant biomarker in carcinogenic processes. An *in vitro* study indicated that  $\beta$ 2-AR could be used as a potential target for cancer therapy [17]. Moreover, it has been reported that the use of  $\beta$ -blockers, particularly propranolol, prolongs survival in breast cancer patients [18, 19].  $\beta$ -blockers are also generally administered to lung cancer patients with coexisting heart diseases. However, little is known about the clinicopathological significance of  $\beta$ 2-AR expression in lung cancer patients and of the relationship between  $\beta$ -blocker use and lung cancer prognosis. Considering previous data on  $\beta$ 2-AR expression within cancer cells,  $\beta$ 2-AR could be an attractive therapeutic target in various human neoplasms, including lung cancer.

Considering this, we conducted a clinicopathological study to evaluate the expression of  $\beta$ 2-AR in patients with NSCLCs. We investigated whether  $\beta$ 2-AR expression was closely associated with post-treatment outcomes and explored the relationship between  $\beta$ 2-AR and various clinicopathological characteristics. Furthermore, we assessed the correlation between  $\beta$ 2-AR expression and both the Ki-67 labeling index (LI), as well as microvessel density (MVD; as determined by CD34).

### Materials and methods

#### Patients

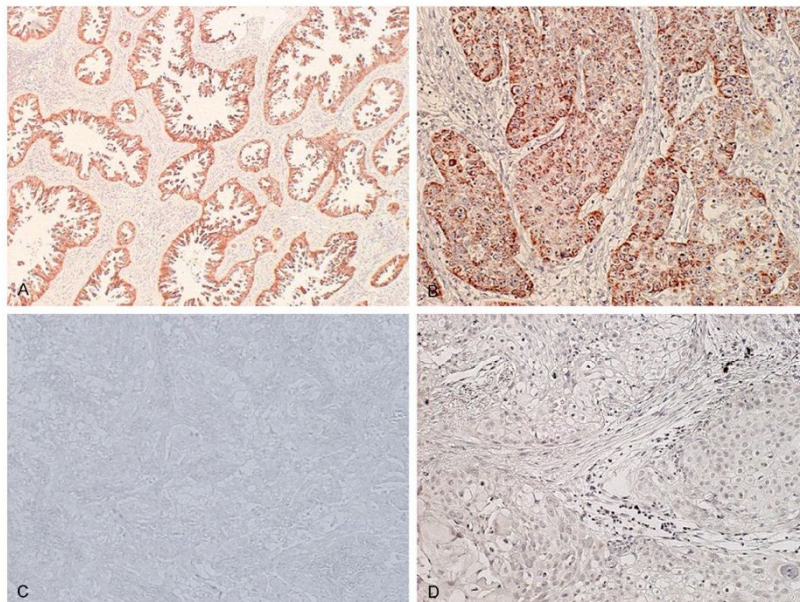
Our study cohort was composed of 347 consecutive patients with NSCLCs (stages I-III), who had undergone resection by lobectomy or pneumonectomy with mediastinal lymph-node dissection at Gunma University Hospital (Maebashi, Gunma, Japan) between June 2003 and December 2010. Of 347 patients, 19 were excluded from further analysis owing to unavailable tissue specimens; thus, 328 patients were enrolled. Our approach to the evaluation and resection of these tumors was identical to the process outlined in our previous study [20]. One patient received platinum-based chemotherapy before surgery as neoadjuvant therapy. In our cohort, 79 patients were administered postoperative adjuvant chemotherapy, with 15

patients receiving platinum-based regimens and 64 patients receiving oral tegafur (a fluorouracil derivative drug). The study protocol was approved by the institutional review board. The tumor specimens were histologically classified according to the World Health Organization criteria. The stages of pathological tumor node metastasis were determined using the International System for Staging Lung Cancer adopted by the American Joint Committee on Cancer and Union Internationale Contre le Cancer [21]. The day of surgery was considered as the first day after the operation. The follow-up duration ranged from 23 to 3,821 days (median, 1,438 days).

#### Immunohistochemical staining

$\beta$ 2-AR expression was determined by immunohistochemical staining using a rabbit anti-human  $\beta$ 2-AR monoclonal antibodies (1:100 dilution; Abcam, Inc., Cambridge, UK) raised against a C-terminal peptide of the human  $\beta$ 2-AR. Immunohistochemical staining was performed on paraffin sections using the polymer peroxidase method (Histofine Simple Stain MAX PO (MULTI) kit; Nichirei Corporation, Tokyo, Japan). Briefly, deparaffinized rehydrated sections were treated with 0.3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. To expose the antigens, sections were autoclaved in 10 mmol/L sodium citrate buffer (pH 6.0) for 5 min, and cooled for 30 min. After rinsing with phosphate-buffered saline, sections were incubated with the anti- $\beta$ 2-AR antibody (1:100) overnight. Thereafter, they were incubated using the Histofine Simple Stain MAX PO (MULTI) kit (Nichirei Corporation). The peroxidase reaction was performed using 0.02% of 3,3'-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in 0.05 M Tris-HCl buffer, pH 7.6. Negative control tissue sections were prepared by omitting the primary antibody. The expression of  $\beta$ 2-AR was considered as positive only if distinct membrane and cytoplasm staining was present. The  $\beta$ 2-AR expression scores were assessed based on the extent of the staining as follows: 1,  $\leq$ 10% of the tumor area stained; 2, 11%-25% of the tumor area stained; 3, 26%-50% of the tumor area stained; and 4,  $\geq$ 51% of the tumor area stained. Tumors in which the stained tumor cells were scored as  $\geq$ 2 were defined as showing positive expression.

## $\beta$ 2-AR expression in NSCLC



**Figure 1.** Immunohistochemical staining of tumor tissues. Immunohistochemical staining sections showed positive or negative  $\beta$ 2 adrenergic receptor ( $\beta$ 2-AR) expression in non-small cell lung cancer. A: Positive  $\beta$ 2-AR expression in adenocarcinoma (AC); B: Positive  $\beta$ 2-AR in squamous cell carcinoma (SCC); C: Negative  $\beta$ 2-AR in AC; D: Negative  $\beta$ 2-AR in SCC.

Mouse monoclonal antibodies against CD34 (1:800 dilution; Nichirei Corporation) and Ki-67 (1:40; Dako, Glostrup, Denmark) were also used. The number of CD34-positive vessels was counted in four randomly selected regions in a 400 $\times$  field (0.26 mm<sup>2</sup> field area). The MVD was defined as the mean number of microvessels per 0.26 mm<sup>2</sup> field area and tumors in which the number of stained tumor cells was greater than the median were defined as areas of high expression. For Ki-67, epithelial cells with nuclear staining of any intensity were considered as positive. Approximately 1,000 nuclei were counted on each tumor slide, and their proliferative activity was assessed as the percentage of Ki-67-stained nuclei (Ki-67 LI) in each sample. The median Ki-67 LI was evaluated, and tumors with an LI greater than the median were considered to show high level of expression. All sections were independently assessed using light microscopy in a blinded manner by at least two of the authors.

### Statistical analysis

*P*-values of less than 0.05 were used to indicate a statistically significant difference. Chi-squared or Fisher's exact tests were used to examine the association between the two categorical variables. The correlation between different variables was analyzed using the non-parametrical Spearman's rank test.

Elderly patients were defined as those older than 65 years, and smokers were defined as those who had smoked at least 100 cigarettes in their lifetimes. Disease staging was divided into two groups: stages I group and stage II/III group. The Kaplan-Meier method was used to estimate survival as a function of time, and differences in survival were analyzed using the log-rank test. Overall survival (OS) was determined as the time from tumor resection to death from any cause. Progression-free survival (PFS) was defined as the time between tumor

## β2-AR expression in NSCLC

**Table 1.** Patient characteristics according to β2-AR expression

Variables	All (n = 328)				AC (n = 222)			non-AC (n = 106)		
	Total (n = 328)	Positive (n = 89)	Negative (n = 239)	P-value	Positive (n = 64)	Negative (n = 158)	P-value	Positive (n = 25)	Negative (n = 81)	P-value
<b>Age</b>										
<65 years/≥65 years	96/232	23/66	73/166	0.405	17/47	59/99	0.125	6/19	14/67	0.559
<b>Sex</b>										
Male/Female	198/130	50/39	148/91	0.344	30/34	74/84	0.996	20/5	74/7	0.149
<b>Smoking</b>										
Yes/No	212/116	57/32	155/84	0.892	32/32	75/83	0.732	25/0	80/1	>0.999
<b>p-Stage</b>										
I/II-III	221/107	52/37	169/70	<b>0.035</b>	40/24	124/34	<b>0.014</b>	12/13	45/36	0.647
<b>Histology</b>										
AC/non-AC	222/106	64/25	158/81	0.318	-	-	-	-	-	-
<b>T factor</b>										
T1/T2-4	151/177	33/56	118/121	<b>0.047</b>	28/36	92/66	<b>0.050</b>	5/20	26/55	0.245
<b>N factor</b>										
N0/N1-2	248/80	59/30	189/50	<b>0.016</b>	42/22	132/26	<b>0.003</b>	17/8	57/24	0.821
<b>Pleural invasion</b>										
Positive/Negative	116/212	37/52	79/160	0.151	23/41	40/118	0.112	14/11	39/42	0.492
<b>Lymphatic permeation</b>										
Positive/Negative	138/190	47/42	91/148	<b>0.016</b>	34/30	44/114	<b>&lt;0.001</b>	13/12	47/34	0.595
<b>Vascular invasion</b>										
Positive/Negative	124/204	44/45	80/159	<b>0.008</b>	31/33	38/120	<b>&lt;0.001</b>	13/12	42/39	0.990
<b>Ki-67</b>										
High/Low	172/156	55/34	117/122	<b>0.038</b>	34/30	54/104	<b>0.009</b>	21/4	63/18	0.502
<b>CD34</b>										
High/Low	168/160	38/51	130/109	0.060	24/40	73/85	0.236	14/11	57/24	0.182

Abbreviations: AC = adenocarcinoma; β2-AR = β2 adrenergic receptor; p-stage = pathological stage. The bold entries show a statistically significant difference.

resection and the first disease progression or death. Multivariate analysis was performed using a stepwise Cox proportional hazards model to identify the independent prognostic factors. Statistical analysis was performed using IBM SPSS Statistics version 21 (IBM corp., NY, USA) for Windows.

### Results

#### *Immunohistochemical analysis and patient demographics*

Three hundred twenty-eight primary NSCLC lesions were analyzed using immunohistochemical analysis. The β2-AR expression was predominantly localized to the plasma membrane (Figure 1). All positive cells showed strong membranous and cytoplasmic staining. Positive expression of β2-AR was identified in 27% (89/328) of all patients; in 29% (64/222) of adenocarcinoma (AC) patients; and in 24% (25/106) of non-AC patients.

The median number of CD34-positive vessels was 12 (range, 1-45) and was chosen as a cut-off point. The median Ki-67 LI was 14% (range, 1-92), and was, likewise, used as a cutoff point. High expressions of CD34 and Ki-67 LI was detected in 51% (168/328) and 52% (172/328) of all patients, respectively.

Non-AC tumors consisted of squamous cell carcinoma (82%, 87/106), adenosquamous carcinoma (4%, 4/106), large-cell carcinoma (3%, 3/106), and large-cell neuroendocrine carcinoma (11%, 12/106).

**Table 1** represents patient characteristics based on the β2-AR expression. In all patients, positive β2-AR expression was significantly associated with disease stage, T factor, N factor, lymphatic permeation, vascular invasion, and Ki-67. This was mirrored in AC patients, where similar association could be seen in between β2-AR expression and almost all of the



## β2-AR expression in NSCLC

**Table 2.** Spearman's rank correlation according to β2-AR

Variables	Total (n = 328)		AC (n = 222)		non-AC (n = 106)		stage I AC (n = 164)	
	r	P-value	r	P-value	r	P-value	r	P-value
Pleural invasion	0.079	0.152	0.107	0.113	0.067	0.497	0.183	<b>0.019</b>
Lymphatic permeation	0.133	<b>0.016</b>	0.240	<b>&lt;0.001</b>	0.052	0.599	0.211	<b>0.007</b>
Vascular invasion	0.146	<b>0.008</b>	0.239	<b>&lt;0.001</b>	0.001	0.990	0.270	<b>&lt;0.001</b>
Ki-67	0.114	<b>0.038</b>	0.175	<b>0.009</b>	0.065	0.507	0.267	<b>0.001</b>
CD34	0.104	0.060	0.079	0.238	0.130	0.185	0.047	0.551

Abbreviations: AC = adenocarcinoma; β2-AR = β2 adrenergic receptor. The bold entries show a statistically significant difference.

aforementioned factors (with the exception of vascular invasion). However, no significant association between β2-AR and any of the tested variables was observed in non-AC patients. Furthermore, in stage I AC patients, positive β2-AR expression was found to be significantly associated with pleural invasion, lymphatic permeation, vascular invasion, and Ki-67 expression (Supplementary Table 1).

### Correlation between β2-AR and different variables

Using Spearman's rank correlation, positive β2-AR expression in all patients was significantly correlated with lymphatic permeation, vascular invasion, and cell proliferation, based on Ki-67 expression levels. When assessing tumors based on their histological type, a statistically significant correlation was observed in AC patients, but not in non-AC patients. In stage I AC patients, a significant correlation with pleural invasion was also identified (Table 2).

### Patient mortality

The 5-year survival rates were 67% for all patients, 73% for AC patients, and 54% for non-AC patients. Table 3 shows the results of univariate and multivariate analyses for each group. Figure 2 shows the Kaplan-Meier survival curve for patients displaying either positive or negative β2-AR expression. In all patients, univariate analysis showed that age, sex, smoking history, disease stage, pleural invasion, lymphatic permeation, vascular invasion, β2-AR, Ki-67, and MVD were significant predictors for worse OS and PFS. Sex was also identified as a significant prognostic factor for OS.

Next, a survival analysis of OS and PFS was performed according to the histological type of

the tumor. Both AC patients, as well as the overall patient cohort, showed identical tendencies for OS and PFS. However, in non-AC patients, disease stage and pleural invasion were significantly associated with poor OS, while smoking and disease stage were significantly associated with poor PFS.

Next, we examined the relationship between β2-AR and survival in patients with stage I AC. Poor OS was significantly associated with age, sex, smoking, pleural invasion, lymphatic permeation, vascular invasion, β2-AR expression, Ki-67 expression, and MVD. These factors were also associated with poor PFS, as was disease stage (Table 4).

Finally, we performed multivariate analyses for each group (Tables 3 and 4) and β2-AR was identified as an independent prognostic marker for predicting a worse PFS in AC patients with stage I disease.

### Discussion

This is the first clinicopathological study to evaluate the prognostic significance of β2-AR expression in patients with NSCLC. Our results demonstrate that a positive β2-AR expression is significantly correlated with lymphatic permeation, vascular invasion, and tumor cell proliferation, and is a promising predictor related to poor outcome in patients with AC. This was particularly true for patients with early-stage AC but was not observed in patients with non-AC. Furthermore, multivariate analyses also identified positive β2-AR expression as an independent prognostic factor for predicting poor PFS in patients with stage I AC. Considering our observations, β2-AR expression seems to play an important role in tumor progression and metastasis. Thus, β2-AR could be a potential

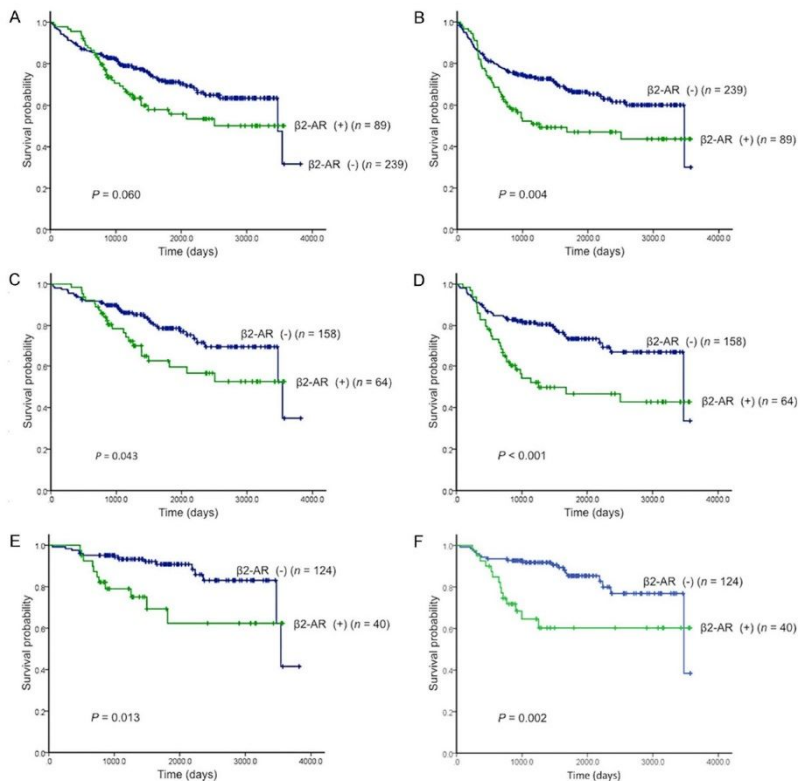
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**Table 3.** Univariate and multivariate analysis of overall survival and progression-free survival

Variables	Overall survival								Progression-free survival							
	Total (n = 328)		AC (n = 222)		Multivariate analysis non-AC (n = 106)		Total (n = 328)		Multivariate analysis AC (n = 222)		Multivariate analysis non-AC (n = 106)					
	5-year survival rate (%)	P-value	5-year survival rate (%)	P-value	HR 95% CI P-value	5-year survival rate (%)	P-value	5-year survival rate (%)	P-value	HR 95% CI P-value	5-year survival rate (%)	P-value				
Age																
<65 years/>65 years	82/61	<b>&lt;0.001</b>	90/65	<b>&lt;0.001</b>		57/54	0.422	75/55	<b>0.001</b>		81/58	<b>0.001</b>	52/50	0.579		
Sex																
Male/Female	62/75	<b>0.003</b>	68/78	<b>0.026</b>	1.282 0.593-2.773 0.527	55/49	<b>0.869</b>	58/65	0.051	0.784 0.471-1.305 0.349	64/68	0.224	0.752 0.390-1.451 0.395	52/40	0.613	
Smoking																
Yes/No	60/79	<b>&lt;0.001</b>	66/80	<b>0.005</b>	1.605 0.736-3.501 0.234	55/0	<b>0.012</b>	54/74	<b>&lt;0.001</b>	2.195 1.244-3.870 0.007	58/74	<b>0.004</b>	2.215 1.129-4.345 0.021	51/0	0.013	
p-Stage																
I/II-III	79/44	<b>&lt;0.001</b>	85/44	<b>&lt;0.001</b>	4.113 2.932-7.701 <b>&lt;0.001</b>	62/44	<b>0.017</b>	74/33	<b>&lt;0.001</b>	3.685 2.568-5.287 <b>&lt;0.001</b>	80/30	<b>&lt;0.001</b>	4.370 2.710-7.046 <b>&lt;0.001</b>	60/38	<b>0.001</b>	
Pleural invasion																
Positive/Negative	47/79	<b>&lt;0.001</b>	49/84	<b>&lt;0.001</b>		46/63	<b>0.021</b>	43/71	<b>&lt;0.001</b>		40/77	<b>&lt;0.001</b>		46/55	0.068	
Lymphatic permeation																
Positive/Negative	50/90	<b>&lt;0.001</b>	48/87	<b>&lt;0.001</b>		51/58	0.432	42/75	<b>&lt;0.001</b>		39/81	<b>&lt;0.001</b>		46/56	0.216	
Vascular invasion																
Positive/Negative	49/78	<b>&lt;0.001</b>	47/86	<b>&lt;0.001</b>		52/56	0.274	44/72	<b>&lt;0.001</b>		37/79	<b>&lt;0.001</b>		52/50	0.479	
β2-AR																
Positive/Negative	56/71	0.062	60/79	<b>0.045</b>	1.125 0.651-1.943 0.674	47/57	0.414	47/66	<b>0.005</b>	1.427 0.982-2.073 0.062	47/74	<b>0.001</b>	1.548 0.955-2.508 0.076	48/52	0.660	
Ki-67																
High/Low	58/76	<b>0.001</b>	60/80	<b>0.004</b>		55/50	0.871	50/72	<b>&lt;0.001</b>		49/75	<b>0.001</b>		50/55	0.730	
CD34																
High/Low	62/73	<b>0.028</b>	66/79	<b>0.013</b>		56/51	0.479	52/70	<b>0.008</b>		52/76	<b>0.006</b>		50/50	0.831	

Abbreviations: AC = adenocarcinoma; β2-AR = β2 adrenergic receptor; 95% CI = 95% confidence interval; HR = hazard ratio; p-stage = pathological stage. The bold entries show a statistically significant difference.

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**Figure 2.** Kaplan-Meier analysis of overall survival (OS) and progression-free survival (PFS) according to  $\beta$ 2-adrenergic receptor ( $\beta$ 2-AR) expression. There was no statistically difference in OS (A), whereas there was a statistically difference in PFS (B) in NSCLC patients with positive  $\beta$ 2-AR expression. A statistically significant difference in OS and PFS was observed in the adenocarcinoma patients with positive  $\beta$ 2-AR expression of stage I-III (OS, C; PFS, D); stage I (OS, E; PFS, F), respectively.

biomarker for early-stage lung adenocarcinoma. However, the reason for the variation in  $\beta$ 2-AR protein expression between AC and non-AC remains unclear. Therefore, further study is warranted to clarify the biological mechanisms underpinning  $\beta$ 2-AR expression in histologically varying tumors.

Previous clinicopathological studies have demonstrated an enhanced expression of  $\beta$ 2-AR in pancreatic cancer, hepatic cellular carcinoma, and oral squamous cell carcinoma [12, 14, 22,

23]. These studies have indicated a close relationship between the enhanced expression of  $\beta$ 2-AR and poor prognosis in pancreatic cancer and hepatic cellular carcinoma, due to a significant correlation between  $\beta$ 2-AR and tumor size and vascular invasion. However, some controversy still remains on whether such a correlation exists in oral squamous cell carcinoma. In our study, the expression level of  $\beta$ 2-AR was significantly correlated with progression and metastasis in AC patients, but no such relationship was found in non-AC patients. The clinico-

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**Table 4.** Univariate and multivariate analysis in overall survival and progression-free survival of stage I AC patients

Variables	Overall survival			Progression-free survival		
	Univariate		Multivariate	Univariate		Multivariate
	5-year survival rate (%)	P-value	HR 95% CI P-value	5-year survival rate (%)	P-value	HR 95% CI P-value
Age						
<65 years/≥65 years	100/77	<b>&lt;0.001</b>		100/69	<b>0.012</b>	
Sex						
Male/Female	76/93	<b>0.001</b>	3.238 1.055-9.944 <b>0.040</b>	77/83	<b>0.032</b>	1.089 0.447-2.651 0.851
Smoking						
Yes/No	77/92	<b>0.003</b>	1.669 0.596-4.678 0.330	71/87	<b>0.003</b>	2.371 0.924-6.087 0.073
p-Stage						
IA/IB	92/71	0.057	1.703 0.764-3.794 0.193	88/62	<b>0.002</b>	2.480 1.229-5.005 0.011
Pleural invasion						
Positive/Negative	59/91	<b>0.001</b>		50/86	<b>&lt;0.001</b>	
Lymphatic permeation						
Positive/Negative	65/90	<b>0.001</b>		53/86	<b>&lt;0.001</b>	
Vascular invasion						
Positive/Negative	45/94	<b>&lt;0.001</b>		46/87	<b>&lt;0.001</b>	
β2-AR						
Positive/Negative	62/91	<b>0.017</b>	1.889 0.839-4.254 0.125	60/85	<b>0.003</b>	2.220 1.077-4.573 0.031
Ki-67						
High/Low	69/90	<b>0.016</b>		63/86	<b>0.002</b>	
CD34						
High/Low	76/90	<b>0.016</b>		64/88	<b>0.004</b>	

Abbreviations: AC = adenocarcinoma; β2-AR = β2 adrenergic receptor; 95% CI = 95% confidence interval; HR = hazard ratio; p-Stage = pathological stage. The bold entries show a statistically significant difference.

pathological parameters involved in the varying expression levels of β2-AR in different histological types, such as adenocarcinoma and squamous cell carcinoma, remain unknown. Therefore, further study is needed to investigate β2-AR expression due to possible mechanical difference based on the histological type of tumor.

β2-AR increases vascularization and enhances the expression of vascular endothelial growth factor (VEGF), MMP2, and MMP9 in ovarian carcinoma [11]. In pancreatic cancer, amplification of the β2-AR signaling pathway might augment the expression of EGFR, VEGF, and MMP2

as a result of variable downstream pathways of β2-AR, such as EGFR pathways and angiogenesis, resulting in the promotion of lymph node metastasis [23]. Other studies have indicated that stimulation of β2-AR induces VEGF, MMP2, and MMP9, followed by the promotion of angiogenesis, cell proliferation, and cell adhesion [24, 25]. Several *in vitro* studies have revealed that the β2-AR agonist isoproterenol promotes the growth of human cancer cells *in vitro* via β2-AR-mediated activation of cAMP/PKA, mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase 1/2 (ERK1/2), and PI3-kinase (PI3 K)/protein kinase B (AKT) signaling pathways. Furthermore, isoproterenol



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activates MAPK/ERK1/2 through a β2-AR-mediated and vascular endothelial growth factor (VEGF)-independent mechanism [26-29]. Thus, β2-AR has recently received attention as a potential therapeutic target for the treatment of cancer.

*In vitro* studies have demonstrated that norepinephrine-induced stimulation of cell proliferation and migration is significantly inhibited by propranolol, a non-cardioselective β2-AR antagonist, in human prostate, colorectal, ovarian, and breast cancer cell lines [30-33]. In addition, propranolol has been shown to suppress prostate cancer cell growth in a nude mouse xenograft model [34]. Moreover, a recent experimental study using melanoma cells has documented that propranolol inhibits cell proliferation and induces apoptosis as a result of regulating the expression of different genes involved in tumor angiogenesis, cell death, or proliferation, such as *TP53*, *Akt3*, *HIF1a*, and *PiK3R5*. It is intriguing, however, that metoprolol, which is a cardioselective β-blocker, hardly affects cell survival or proliferation [35]. Furthermore, other experimental studies have shown that, after encouraging their proliferation, lung cancer cell lines produced noradrenaline, and propranolol inhibited their growth due to restricting the activity of p-CREB and p-ERK1/2 downstream signaling pathways [36]. Other *in vivo* studies have revealed that β-blockers could augment the effectiveness of chemotherapy in certain conditions [37].

Clinical reports have shown that the β-blocker propranolol, but not atenolol, which is a β1-adrenergic receptor-selective antagonist, improves the outcome for breast cancer patients by reducing metastasis, recurrence, and mortality. Reports also indicate that angiotensin receptor blockers and angiotensin converting enzyme inhibitors do not affect patient prognosis in breast and prostate cancer patients [38, 39]. However, in those studies, the effect of β2-AR expression on patient prognosis was not adequately examined. For example, it has been demonstrated that strong β2-AR expression in breast cancer patients can be an indicator good early prognosis due to effects of tamoxifen, an estrogen antagonist, but after 60 months, this trend is reversed [40]. Therefore,

it is also important to evaluate the β2-AR expression in lung adenocarcinoma patients.

Although adjuvant chemotherapy including cisplatin-based combination regimens after surgery is the current standard of care based on the results of phase III trials in patients with stage II/III NSCLCs, the use of adjuvant chemotherapy with cisplatin-based regimens in AC patients with stage I disease remains controversial [41-43]. Therefore, an investigation of the possible markers capable of determining the candidates for adjuvant therapy is extremely relevant for treating patients with stage I lung adenocarcinoma. In light of our data, propranolol may potentially be used as a novel post-surgery adjuvant therapy for early-stage lung adenocarcinoma patients showing positive β2-AR expression.

However, there were several limitations to our study. First, we used a single-center cohort, which may have biased our results. Second, it remains unclear what precisely is the optimal cutoff point for the expression level of β2-AR. Further study is warranted to investigate and identify the appropriate cutoff level for β2-AR. Third, the detailed mechanism responsible for β2-AR expression in various neoplasms still remains unclear. To the best of our knowledge, our investigation is the first study to examine the expression of β2-AR in patients with lung cancer. Keeping this in mind, future studies should aim to clarify the clinicopathological problems involved, such as the appropriate cut off level for β2-AR, before β2-AR inhibition would be truly applicable in clinical settings.

In conclusion, the expression of β2-AR is closely related to tumor cell proliferation, angiogenesis, and metastasis, and is a significant independent marker for predicting a worse outcome in patients with AC, particularly those with stage I disease. Our results suggest that β2-AR can play a crucial role in the development of tumor progression and metastasis. Therefore, the inhibition of β2-AR by antagonists such as propranolol may be a future potential target for anticancer therapy in early-stage lung adenocarcinoma.

### Disclosure of conflict of interest

None.

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