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Original Article

ALKBH5 gene is a novel biomarker that predicts the prognosis of pancreatic cancer: A retrospective multicohort study

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Backgrounds/Aims: Discovery of new prognostic factors for cases in which the pancreatic cancer scoring and staging system does not result in a clear definition is imperative. We examined the role of Human AlkB homolog H5 (*ALKBH5*) as a prognostic marker for pancreatic cancer. **Methods:** Patient data were extracted from the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA). The prognostic value of *ALKBH5* was confirmed via analysis of *ALKBH5* and other clinical factors, such as age, sex, and stage, using the time-dependent area under the curve (AUC) of Uno's C-index, the AUC value of the receiver operating characteristics (ROC) at three years, the Kaplan-Meier survival curve, and multivariate analysis. **Results:** *ALKBH5* showed excellent prognosis prediction in comparison with existing markers in the two independent cohorts (n=262). Kaplan-Meier survival analysis showed that *ALKBH5* expression was positively associated with overall survival (log-rank test, ICGC, p=0.001; TCGA, p=0.01). Notably, comparison of C-index and AUC values in ROC analysis showed that *ALKBH5* was associated with high C-index and AUC values compared with other clinical variables (C-index: ICGC, 0.621; TCGA, 0.614 and AUC at three years: ICGC, 0.609; TCGA, 0.558). Multivariate analysis demonstrated that *ALKBH5* is an independent prognostic factor (ICGC, p=0.0123; TCGA, $p<0.001$). **Conclusions:** These findings contribute to the study of RNA methylation in pancreatic cancer. We believe that *ALKBH5* is a new prognostic marker for pancreatic cancer. **(Ann Hepatobiliary Pancreat Surg 2018;22:305-309)**

Key Words: ALKBH5; RNA methylation; Pancreatic cancer; Prognosis

INTRODUCTION

Pancreatic neoplasms are one of the few cancers with a mortality rate approaching 100%.[1] Furthermore, only 15-20% of patients are diagnosed during early stages because of nonspecific symptoms and approximately 50% present with distant metastasis at the time of diagnosis.[2,3] As the etiology and screening test for this highly lethal disease have yet to be well defined, it is important to identify genetic factors that contribute to the development of this cancer.[4]

N^6-methyladenosine (m^6A) is the most prevalent epitranscriptomic modification of the mRNA of higher eukaryotes.[5,6] Human AlkB homolog H5 (*ALKBH5*) is a demethylase that oxidatively reverses several mRNAs, and significantly affects mRNA export and RNA metabolism, as well as the assembly of mRNA processing factors in nuclear speckles. This modification plays an essential role in the regulation of mRNA translation and RNA metabolism. Evidence strongly suggests that epigenetic alterations, especially methylation induce tumorigenesis- associated cellular changes, suggesting the role of oncogenic mechanisms beyond DNA mutations in cancer.[6-9]

The methylation of related genes and mRNA has been strongly linked to the development and progression of pancreatic cancer. The regulation of the expression of oncogenic and tumor suppressor genes by demethylation or methylation mechanisms plays a significant role in identi-

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fied tumorigenesis. Thus, studies investigating epigenetic changes associated with pancreatic cancer are very important for the development of new diagnostic and therapeutic methods.

Based on the function of *ALKBH5* as an mRNA methylation regulator, we hypothesized that *ALKBH5* influences the survival of patients with pancreatic cancer. Therefore, we examined the role of *ALKBH5* as a novel prognostic marker complementing the current deficient staging and scoring systems.

MATERIALS AND METHODS

Data acquisition and characteristics

Primary and processed data, including mRNA expression and clinical information, were downloaded from The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) in October 2017.[10-12] These processes were performed using the *cgdsr* package of R software version 3.5.0 (The R Foundation for Statistical Computing, 2018). The flow of this study described in Fig. 1.

Survival analysis

Survival analyses were performed to predict the overall survival (OS) using three methods: [1] Kaplan-Meier survival curves to evaluate the accuracy of the discrimination, [2] Uno's C-index for the time-dependent area under the curve (AUC) analysis, and [3] AUC values in receiver operating characteristics (ROC) at three years. These analyses were performed using R packages *survival* and *survAUC*. C-indices and AUC values ≥ 0.6 were considered acceptable for survival predictions. Using Kaplan-Meier analyses, we determined the optimal cutoff value with the maximal Uno's C-index by 5-fold cross-validation. We used univariate and multivariate Cox regression to compare the effect of *ALKBH5* on prognosis along with other clinical variables. Statistical analyses were performed using R software.

RESULTS

Patient distribution

In total, 172 TCGA and 90 ICGC patients were included in this study. Of the 172 patients in the TCGA

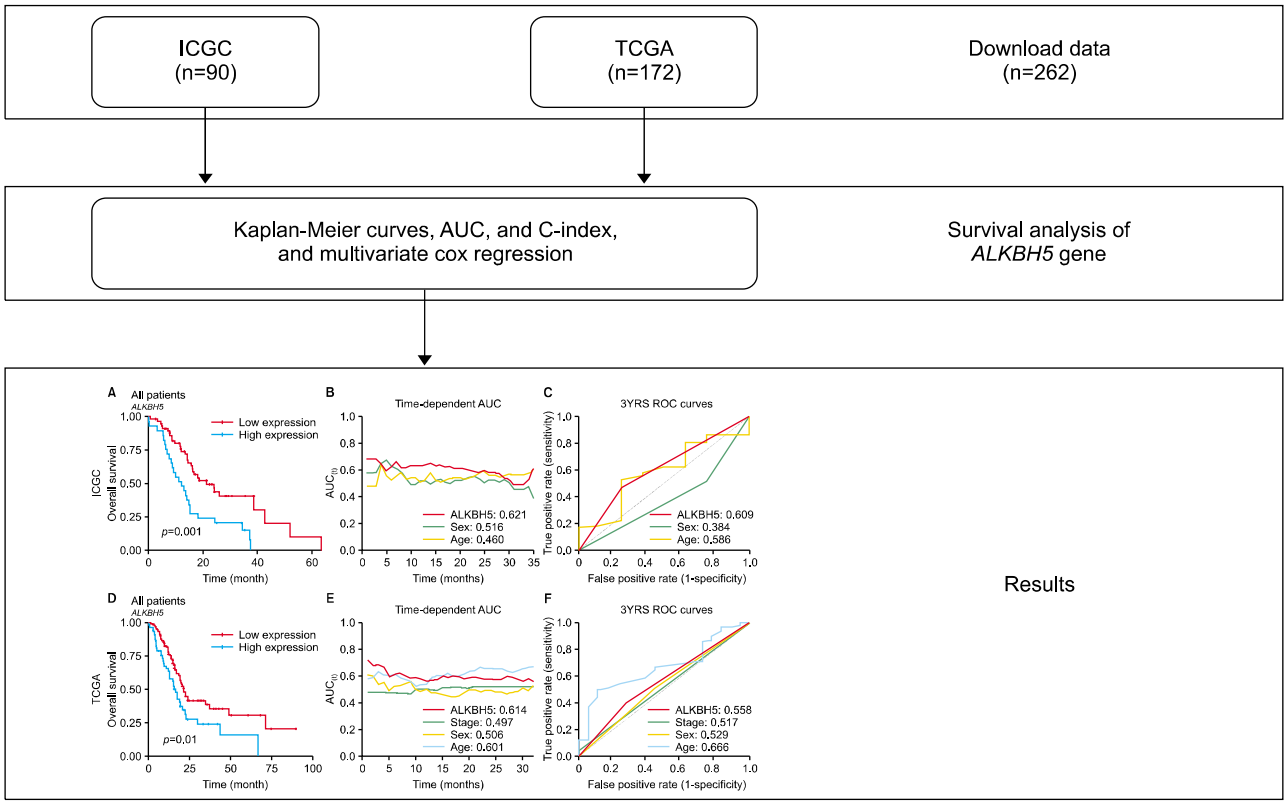

Fig. 1. Overview of the study workflow.

cohort, 112 showed high *ALKBH5* expression values and 60 had low *ALKBH5* expression values. The average age at diagnosis of TCGA and ICGC was 64.7 years and 65.9 years, respectively. Of the 90 patients in the ICGC cohort, 61 showed high and 29 low *ALKBH5* expression. Patient information used in the current study is listed in Table 1.

ALKBH5 survival curve

To identify the discriminatory power of *ALKBH5* as a categorical variable, we analyzed Kaplan-Meier curves for *ALKBH5* gene expression and survival. High expression of *ALKBH5* was a significant predictor of overall survival in the TCGA and ICGC cohorts (p=0.01 and p=0.001, Fig. 2A, D). The prognostic value was further confirmed using multivariate analysis (ICGC, p=0.0123; TCGA, p $<$0.001, Table 2).

Table 1. Patients' information used in current research in the TCGA and ICGC cohorts

	Group	ICGC	TCGA
ALKBH5	All patients	90	172
	High expression (event)	61 (32)	112 (54)
	Low expression (event)	29 (26)	60 (38)
Patients' information	Male	47	94
	Female	43	78
	Stage I & II	-	164
	Stage III & IV	-	8

ALKBH5 C-index and AUC values

To evaluate the prognostic value of *ALKBH5*, we compared this gene with other clinical factors, such as age, stage, and sex, in both cohorts. We analyzed gene expression values as continuous variables using Uno's C-indices and AUC values at three years. *ALKBH5* showed high C-index values in the two independent cohorts compared with the other factors (TCGA: 0.614, ICGC: 0.621, Fig. 2B, E). The three-year AUC value was also significantly higher than that of the other factors across the two cohorts (ICGC, 0.609; TCGA, 0.558, Fig. 2C, F).

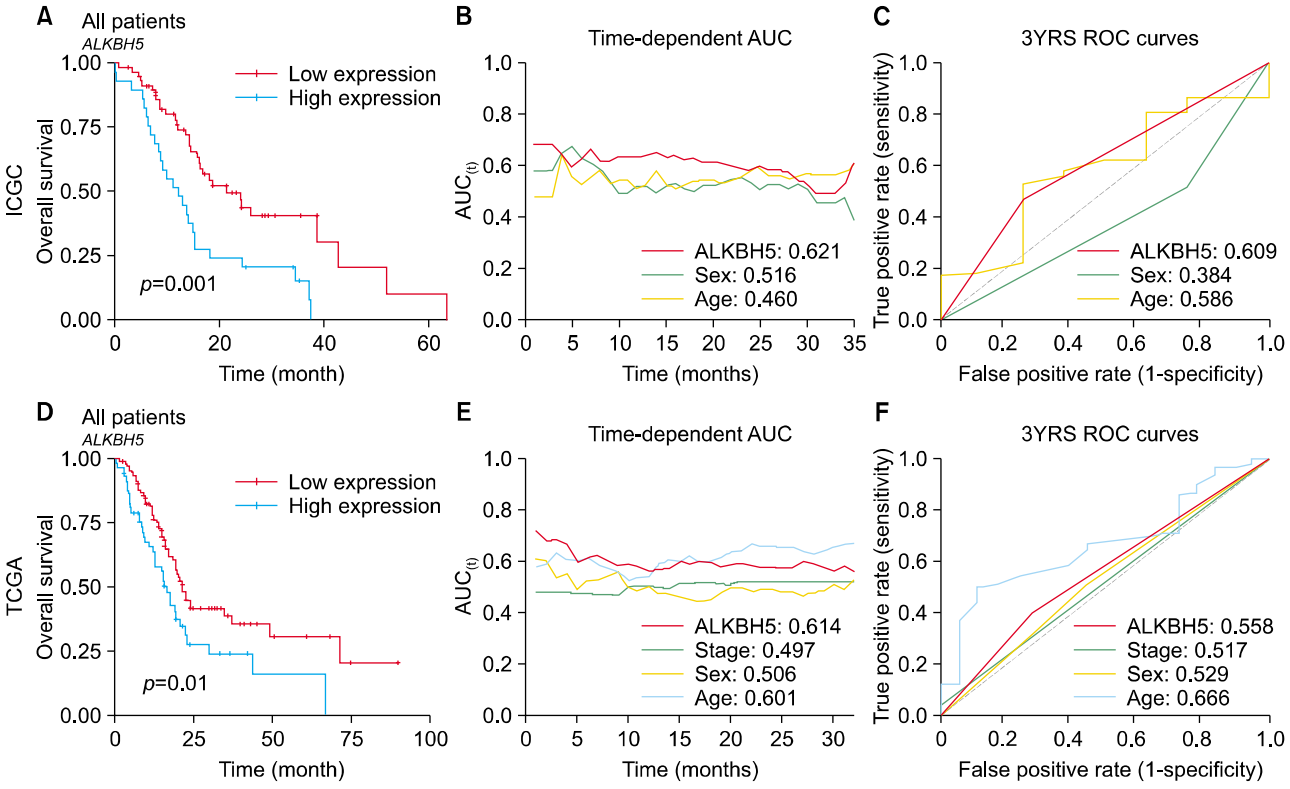

Fig. 2. Survival analyses of the ICGC and TCGA cohorts. (A, D) Kaplan-Meier estimates of overall survival of pancreatic cancer patients according to *ALKBH5* gene expression. (B, E) Time dependent area under the curve (AUC) according to *ALKBH5* gene expression. (C, F) Receiver operating characteristic (ROC) curve at three years according to *ALKBH5* gene expression.

Table 2. Univariate and multivariate analysis of overall survival in each cohort

Parameters	Univariate analysis				Multivariate analysis			
	p	HR	95 Cl		*p*	HR	95 Cl	
ICGC								
ALKBH5	0.00146**	0.4187	0.2449	0.7157	＜0.001***	0.3974	0.2305	0.685
Sex	0.421	1.24	0.7335	2.097	0.251	1.4027	0.7871	2.500
Age	0.881	0.9979	0.9702	1.026	0.9429	1.0011	0.9709	1.032
TCGA								
ALKBH5	0.011*	0.5812	0.3826	0.8829	0.0123*	0.5847	0.3841	0.8902
Stage	0.716	0.8072	0.2545	2.561	0.5214	0.6846	0.2150	2.1799
Sex	0.39	0.8354	0.5543	1.259	0.4164	0.8426	0.5575	1.2735
Age	0.0136*	1.0261	1.005	1.047	0.0119*	1.027	1.0058	1.0480

DISCUSSION

In this study, we investigated the survival rate of patients diagnosed with pancreatic cancer based on the *ALKBH5* gene expression using TCGA and ICGC databases. Patients in the two cohorts were comparable in age and sex but not significantly. As ICGC is an ongoing project, additional patient information will be released in the future. Survival analysis demonstrated that high *ALKBH5* expression showed good discriminatory power compared with age, sex, and stage.

Less than 10% of patients diagnosed with pancreatic cancer present with resectable status and the overall 5-year survival is ＜4%.[3,13] Patients with small, surgically resectable cancers have a realistic chance of a cure and exhibit 5-year survival rates of approximately 20-30%.[3,13-16] Although many studies tried to predict the prognosis of patients with pancreatic cancer based on clinical and genetic variables, CA-19-9 is the only clinical biomarker approved by the United States Food and Drug Administration.[17,18] The poor prognosis of pancreatic cancer is attributed to its late presentation and lack of accurate predictive biomarkers for early diagnosis and prognosis.[19] In the current study, *ALKBH5* facilitated the classification of patients into two risk groups in two independent cohorts. In addition, it also scored higher C-index compared with stage and age that are important prognostic factors. In particular, the AUC value of *ALKBH5* remained constant regardless of the follow-up time, which provides a stable prediction of prognosis of the patient.

The past several years have witnessed a vast increase in our knowledge regarding epigenetic features in human cancers.[20,21] M6A RNA methylation is regulated by several molecules such as methylases (METTL3, METTL14, WTAP) and demethylases (FTO, *ALKBH5*)[20-23] that act as oncogenes and/or tumor suppressor genes in various cancers. High expression of *ALKBH5*, a well-known m6A demethylase, leads to oncogenic progression in glioblastoma and breast cancer by stabilizing FOXM1, NANOG, and KLF4 mRNA. In breast cancer, hypoxia in the cancer progenitor cells induces high expression of *ALKBH5* via activation of hypoxia-inducible factors. Thus, the m6A modification level is reduced, which increases the stability and expression of NANOG and KLF4, which are involved in the development of breast cancer stem cells. In the case of glioblastoma, m6A modification modulates methylases such as METTL3 or METTL14, which increases the expression of oncogenes such as KLF4. *ALKBH5* regulates FOXM1 gene expression and the altered expression affects GSC tumorigenesis via the FOXM1 axis.[20-23] The discovery of new biomarkers via elucidation of these RNA methylation mechanisms is expected to facilitate pancreatic cancer diagnosis and prognosis by complementing the current chromosomal and nucleotide methods.

With increasing evidence suggesting the role of epigenetic alterations especially methylation in triggering tumorigenesis-associated cellular changes, the field of cancer research has evolved to incorporate oncogenic mechanisms beyond DNA mutations.In particular, the importance of m6A in mRNA has attracted attention due to its role in epitranscriptomic modification of cellular differentiation and pluripotency. However, the effect of modification of m6A mRNA on the complex process of tu-

morigenesis is unclear.

In conclusion, we found that the survival rate of patients with pancreatic cancer was enhanced under high *ALKBH5* expression. Despite the unclear mechanism of action of *ALKBH5* in tumorigenesis, it has been suggested that *ALKBH5* may have a positive effect on the development and progression of pancreatic cancer. We believe that these findings will contribute to the study of RNA methylation in pancreatic cancer.

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