Long non-coding RNA BACE1-AS is an independent unfavorable prognostic factor in liver cancer

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Abstract. Liver cancer is one of the leading causes of cancer-associated deaths with incidence rates continuously on the rise. Biomarkers are urgently required for early diagnosis and better prognostic classification, which is essential for risk stratification and optimizing treatment strategies in clinical settings. By analyzing the data extracted from The Cancer Genome Atlas database using R, the long noncoding RNA (lncRNA) β-site APP-cleaving enzyme 1 antisense (*BACE1-AS)* was discovered to have both high diagnostic and prognostic values in liver cancer, which could serve as a promising biomarker in clinical settings. Precisely, lncRNA *BACE1-AS* is significantly overexpressed in liver cancer and its levels vary within different subgroups, suggesting its tumorigenic role. Furthermore, higher *BACE1-AS* predicts poorer overall survival and relapse-free survival outcomes. Overall, the present study demonstrated that *BACE1-AS* may be involved in liver cancer progression and could serve as a promising biomarker for diagnosis and prognostic evaluation.

Introduction

Liver cancer is the sixth most commonly diagnosed cancer worldwide and ranks among the top four leading causes of cancer-associated deaths in 2018 (1). It is estimated that both incidences and deaths caused by liver cancer will increase in the United States during the next ten years, resulting in liver cancer becoming the third leading cause of cancer-associated mortality by 2030 (2). Although ultrasound and optional combination of alpha-fetoprotein (AFP) testing have enabled the regular screening of liver cancer among at-risk individuals (3), biomarkers are urgently required for early diagnosis and better prognostic classification; which is essential for optimal treatment strategies (4,5).

β-site APP-cleaving enzyme 1 (BACE1) is a key β-secretase enzyme that initiates the formation of β-amyloid (Aβ) peptide, which is the central player in the pathogenesis of Alzheimer's disease (AD) (6). The expression levels, and the enzymatic activities of BACE1 protein, as well as *BACE1* SNP, have been reported to be associated with specific clinical features, for example, patients with Alzheimer's disease tend to have higher brain BACE1 levels compared with normal controls (7-9). A long non-coding RNA (lncRNA) *BACE1* antisense (*BACE1-AS*, also known as *BACE1-AS1*) was identified in 2008 as a regulator of *BACE1* expression by increasing *BACE1* mRNA stability, and whose deregulation is crucial in AD (10). Although *BACE1-AS* is universally expressed in various tissues including in malignancies, such as ovarian cancer (11), its functions in cancer have thus far remained largely unknown (11,12).

Based on data extracted from The Cancer Genome Atlas (TCGA) database, the present study investigated the roles of *BACE1-AS* in a liver cancer cohort. It was found that *BACE1-AS* is highly expressed in liver cancer and that *BACE1-AS* expression is an independent prognostic factor of overall survival (OS) and relapse-free survival (RFS) in patients with liver cancer.

Materials and methods

TCGA data mining. The RNA-Seq expression data and clinical information of patients (mean average=61 years; range, 16-90 years) with liver cancer were downloaded and based upon data generated by TCGA Research Network: https://www.cancer.gov/tcga. A total of 371 patients were included for the study including 121 female and 250 male patients. RNA-Seq by Expectation-Maximization (RSEM) (13) was used for accurate transcript abundance quantification, and the resulting

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values were used for subsequent statistical analysis. The age cut-off was set as 55: Young (aged <55 years) and old patients (aged ≥55 years). The grading system used was Edmondson grade (14). The TNM staging system refers to the newest NCCN guidelines (15).

Statistical analysis. All statistical analyses were performed using R (version 3.5.1) (16). Differential expression within a category was analyzed using nonparametric Wilcoxon rank sum test and Kruskal-Wallis test, depending on the numbers of variables tested. Receiver-operating characteristic curve (ROC) was drawn by the pROC package to evaluate the diagnostic capability, and Youden's J index was used for determining the threshold value for dividing patients into *BACE1-AS* high and *BACE1-AS* low groups. Fisher's exact or Pearson's χ^2 test was applied to study the association between *BACE1-AS* expression and the clinical characteristics of patients. Survival analysis was performed with Kaplan-Meier curves using the survival package in R (17); the statistical significance was assessed using the log-rank test. Univariate and multivariate Cox regression analyses were performed using Cox proportional hazard models. Data visualization was performed using the ggplot2 package in R (18). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patients' characteristics. A total of 370 patients along with their RNA-Seq data were included for analysis in the present study. The patients were followed up for ten years and their information, such as histological types, stages and vital status were summarized and detailed in Table I.

BACE1-AS is overexpressed in liver cancer and the levels of which varies among different subgroups. The abundances of *BACE1-AS* transcript were analyzed in all patients included in the present study and were further compared within different categories (Fig. 1). *BACE1-AS* was highly elevated in liver cancer tissues compared with healthy liver tissues ($P<2.210^{-16}$). Furthermore, a significant difference was observed among patients with different histological grades, with a trend of higher levels of expression corresponding to advanced histological grades ($P=7.910^{-6}$). The analysis of patients at different tumor stages also presented with the aforementioned trend, with an exception at stage IV where the levels of *BACE1-AS* displayed a sudden fall (P=0.0079). A similar trend was also observed in the tumor size staging classification subgroups (T classification; P=0.017). Notably, when patients were divided according to histological types, there was a trend of higher levels of *BACE1-AS* in the mixed hepatocellular carcinoma (HCC)/hepatocellular cholangiocarcinoma (CAA) compared with HCC alone; the P-value indicated a near-significant trend overall (P=0.063).

Accessing the diagnostic capability of BACE1-AS. To further verify the aforementioned findings, the ROC curves were plotted to evaluate the diagnostic ability of *BACE1-AS* as a biomarker (Fig. 2). Consistent with the aforementioned results, *BACE1-AS* showed both high sensitivity and specificity when differentiating tumors from healthy tissues. The sensitivity and specificity was 0.94 and 0.836, respectively, with an area under the curve (AUC) value of 0.949, demonstrating high differential diagnostic potential. The AUC remained high when healthy individuals were compared with patients with cancer of different clinical stages (AUCs:, 0.933 for stage I; 0.967 for stage II; 0.964 for stage III; and 0.908 for stage IV), which indicates *BACE1-AS* as a good diagnostic marker of liver cancer, regardless of the tumor stage. In order to simplify the subsequent analysis, Youden's J statistic was calculated to determine the optimal cut-off point of *BACE1-AS* expression (1.650), which was subsequently used to divide patients with liver cancer into two groups: *BACE1-AS* high group and *BACE1-AS* low group.

Associations between BACE1-AS expression levels and clinicopathological parameters of patients with liver cancer. The expression of *BACE1-AS* was significantly associated with patients' histological grades, clinical stages and tumor (T) classification (Table II). This was consistent with the aforementioned results. Notably, a modest but significant association was found between tumor histological types and *BACE1-AS* levels (P=0.043). Furthermore, the age of patients was associated with *BACE1-AS*, with younger patients (aged <55 years) presenting with higher levels of *BACE1-AS* (P=0.001).

High expression of BACE1-AS predicts poorer OS in patients with liver cancer. To verify the prognostic value of *BACE1-AS* in patients with liver cancer, Kaplan-Meier curves were generated (Fig. 3). Log-rank test was used for comparison between groups. The *BACE1-AS* high group had a significantly lower OS time compared with the *BACE1-AS* low group (P=0.00062). Subsequently, the prognostic value of *BACE1-AS* within different subgroups was studied. *BACE1-AS* remained a negative prognostic factor in tumors of advanced clinical stages (stage III/IV) and tumors of advanced histopathology stages (G3/G4) (P=0.0061 and P=0.0031, respectively). The aforementioned trend was not observed in tumors of lower stages (stage I/II). High *BACE1-AS* expression was a poorer prognostic marker in male patients (P=0.00011), while no such significance was detected in females. Meanwhile, *BACE1-AS* was a poor prognostic marker both in young (aged <55 years) and old patients (aged ≥55 years).

In line with the aforementioned data, univariate Cox regression analysis (Table III) showed that patients with high *BACE1-AS* expression had a significantly shorter OS time (P=0.001; HR, 1.81; 95% CI, 1.28-2.56). Furthermore, other prognostic parameters were also analyzed and clinical stage, T classification and residual tumor were identified as negative prognostic factors. Based on these results, multivariate Cox regression analysis was applied to validate the four established factors, which were all revealed be significant prognostic factors except clinical stage. Thus, *BACE1-AS* is an independent prognostic factor in liver cancer; specifically, the adjusted HR was 1.76 (95% CI, 1.24-2.49).

The upregulation of BACE1-AS predicts poorer RFS in liver cancer cells. Subsequently, the role of *BACE1-AS* in the

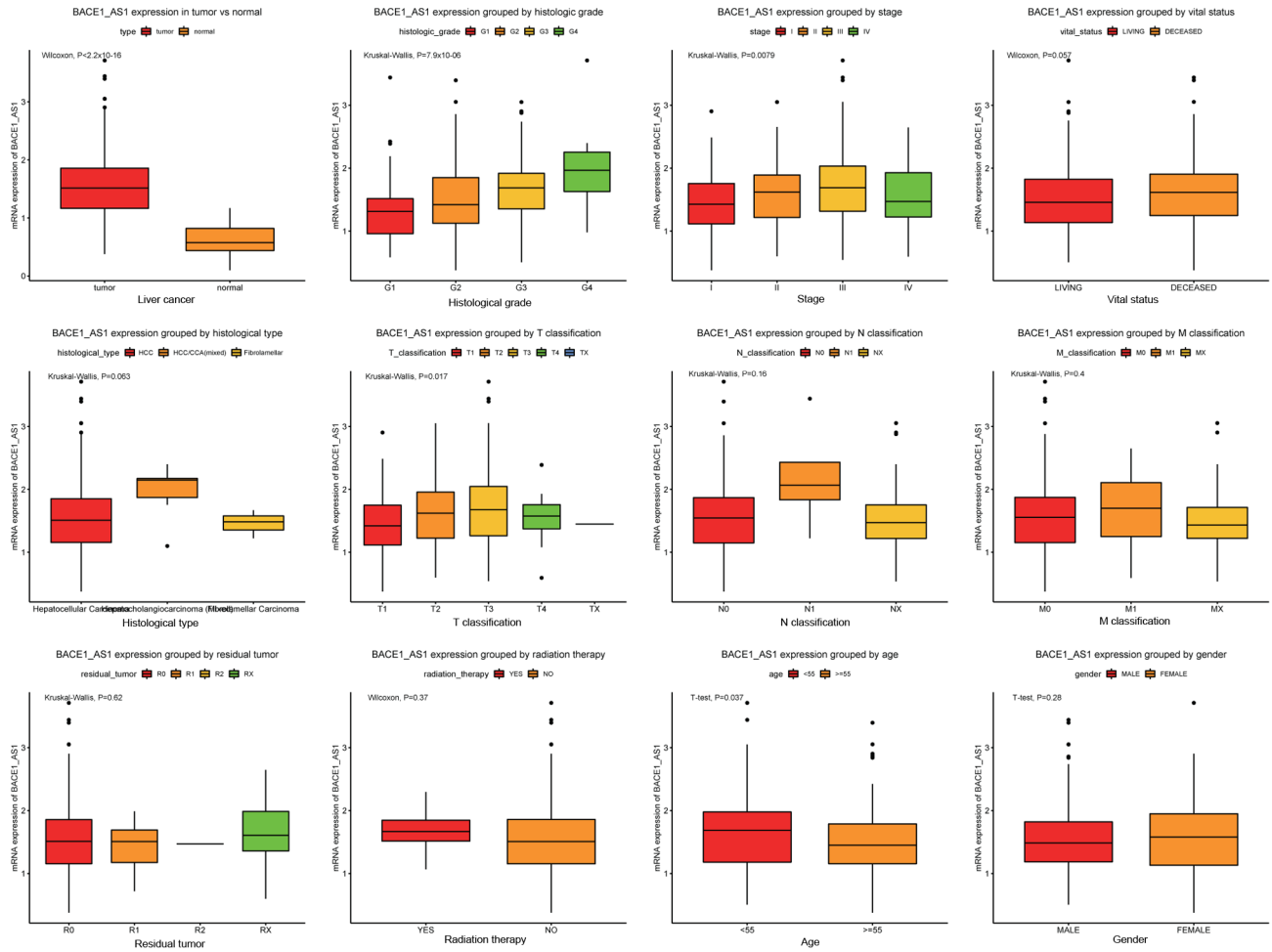

Figure 1. BACE1-AS is overexpressed in liver cancer and is differentially expressed in various subtypes. The significance was calculated based on nonparametric Wilcoxon and Kruskal-Wallis tests. The subgroups include tumors vs. healthy liver tissue, histological grades, stages, vital status, histological types, T classification, N classification, M classification, residual tumor, radiation therapy, age and sex. BACE1-AS, β-site APP-cleaving enzyme 1 antisense.

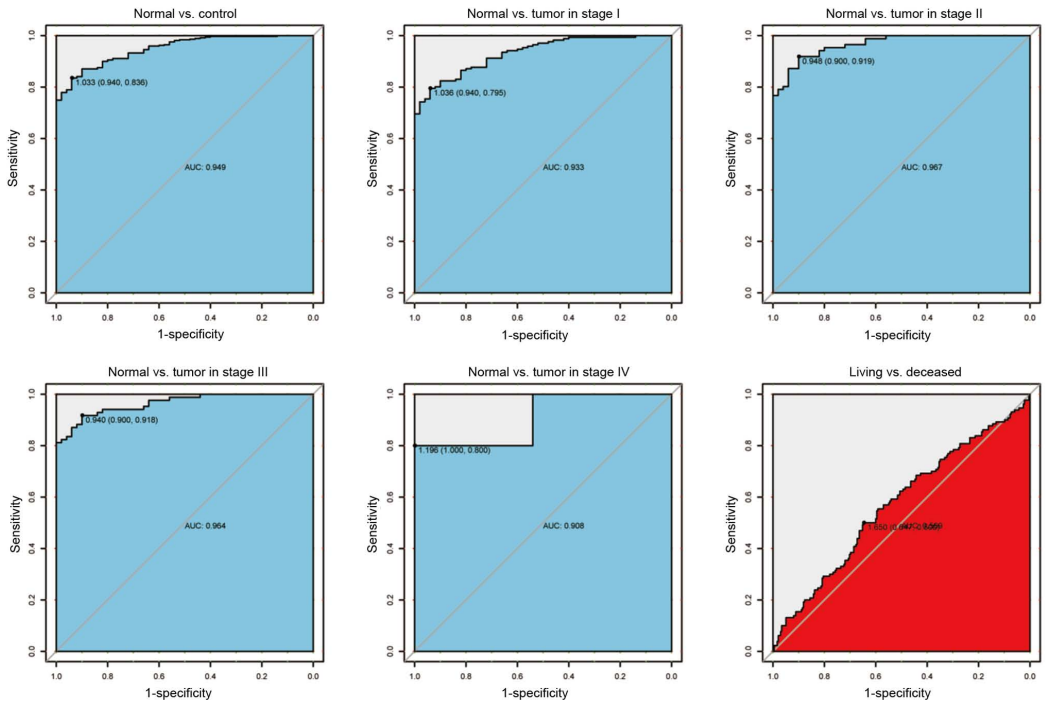

Figure 2. The receiver operating characteristics curve of BACE1-AS in liver cancer cohorts and different stages. AUC, area under the curve; BACE1-AS, β-site APP-cleaving enzyme 1 antisense.

Table I. Clinical characteristics of the patients included in the present study.

Characteristics	Number of patients (%)
Age, years	
<55	117 (31.54)
≥55	253 (68.19)
NA	1 (0.27)
Sex	
Female	121 (32.61)
Male	250 (67.39)
Histological type	
Fibrolamellar carcinoma	3 (0.81)
Hepatocellular carcinoma	361 (97.3)
Hepatocholangiocarcinoma	7 (1.89)
Edmondson grade	
G1	55 (14.82)
G2	177 (47.71)
G3	122 (32.88)
G4	12 (3.23)
NA	5 (1.35)
TNM stage	
I	171 (46.09)
II	86 (23.18)
III	85 (22.91)
IV	5 (1.35)
NA	24 (6.47)
T classification	
T1	181 (48.79)
T2	94 (25.34)
T3	80 (21.56)
T4	13 (3.5)
TX	1 (0.27)
NA	2 (0.54)
N classification	
N0	252 (67.92)
N1	4 (1.08)
NX	114 (30.73)
NA	1 (0.27)
M classification	
M0	266 (71.7)
M1	4 (1.08)
MX	101 (27.22)
Radiation therapy	
No	338 (91.11)
Yes	8 (2.16)
NA	25 (6.74)
Residual tumor	
R0	324 (87.33)
R1	17 (4.58)
R2	1 (0.27)
RX	22 (5.93)
NA	7 (1.89)
Vital status	
Deceased	130 (35.04)
Living	241 (64.96)
Relapse	
No	179 (48.25)
Yes	139 (37.47)
NA	53 (14.28)
BACE1-AS1	
High	153 (41.24)
Low	218 (58.76)

BACE1-AS, β-site APP-cleaving enzyme 1 antisense; TNM, Tumor-Node-Metastasis; NA, not applicable; R0, microscopic completely removed; R1, microscopic residual; R2, macroscopic residual.

prediction of RFS was analyzed (Fig. 4). Patients expressing higher levels of *BACE1-AS* had a significantly shorter RFS time compared with patients with lower *BACE1-AS* (P=0.0028). Subgroup analysis indicated that *BACE1-AS* expression was a negative predictor in liver cancer for both lower and advanced histopathological grades (G1/G2 and G3/G4, respectively). For clinical stage, *BACE1-AS* expression was associated with shorter RFS time in patients with stage I/II, whereas patients with advanced cancer (stage III/IV) were unaffected. Consistent with the OS analysis, *BACE1-AS* retained its prognostic ability in male patients (P=0.00098), which was not observed in female patients. Moreover, the expression of *BACE1-AS* predicted shorter RFS time in younger patients (aged <55 years) (P=0.037), whereas no prognostic potential was demonstrated in older patients (aged ≥55 years).

Univariate Cox regression analysis revealed *BACE1-AS,* tumor stage, T classification and residual tumor as prognostic factors (Table IV). Furthermore, multivariate Cox analysis identified *BACE1-AS* expression, T classification and residual tumor as independent predictive factors for RFS, and the adjusted HR for *BACE1-AS* expression was 1.58 (95% CI, 1.13-2.22).

Discussion

The increasing liver cancer incidence and liver cancer-associated mortality warrants the discovery of new biomarkers both for early diagnosis and improved treatment surveillance. Previous studies have discovered a few biomarkers that can be used as potential diagnostic and prognostic markers (19-21). It has recently been shown that *BACE1-AS*, an antisense lncRNA of *BACE1* frequently discussed in AD, is also involved in tumors, particularly as a tumor suppressor (11,12). The present study demonstrated, using the TCGA database, that *BACE1-AS* was highly elevated in liver cancer, which was significantly associated with tumor grade and staging. Besides,

Table II. Associations between the clinicopathologic variables and BACE1-AS expression.

Clinical characteristics	Variable	BACE1-AS1 expression			χ^2	P-value
		No. of patients	High, n (%)	Low, n (%)		
Age	<55	117	63 (41.45)	54 (24.77)	10.7607	0.001
	≥55	253	89 (58.55)	164 (75.23)		
Sex	Female	121	58 (37.91)	63 (28.9)	2.9231	0.087
	Male	250	95 (62.09)	155 (71.1)		
Histological type	Fibrolamellar	3	1 (0.65)	2 (0.92)	5.8857	0.040
	Hepatocellular	361	146 (95.42)	215 (98.62)		
	Hepatocholangiocarcinoma	7	6 (3.92)	1 (0.46)		
Histologic grade	G1	55	11 (7.24)	44 (20.56)	28.2803	0.000
	G2	177	64 (42.11)	113 (52.8)		
	G3	122	68 (44.74)	54 (25.23)		
	G4	12	9 (5.92)	3 (1.4)		
Stage	I	171	58 (39.46)	113 (56.5)	10.3777	0.011
	II	86	42 (28.57)	44 (22)		
	III	85	45 (30.61)	40 (20)		
	IV	5	2 (1.36)	3 (1.5)		
T classification	T1	181	60 (39.22)	121 (56.02)	11.3008	0.015
	T2	94	46 (30.07)	48 (22.22)		
	T3	80	41 (26.8)	39 (18.06)		
	T4	13	6 (3.92)	7 (3.24)		
	TX	1	0 (0)	1 (0.46)		
N classification	N0	252	111 (72.55)	141 (64.98)	5.0198	0.079
	N1	4	3 (1.96)	1 (0.46)		
	NX	114	39 (25.49)	75 (34.56)		
M classification	M0	266	119 (77.78)	147 (67.43)	5.2756	0.055
	M1	4	2 (1.31)	2 (0.92)		
	MX	101	32 (20.92)	69 (31.65)		
Radiation therapy	No	338	138 (96.5)	200 (98.52)	0.7519	0.386
	Yes	8	5 (3.5)	3 (1.48)		
Residual tumor	R0	324	133 (88.67)	191 (89.25)	1.6516	0.771
	R1	17	6 (4)	11 (5.14)		
	R2	1	0 (0)	1 (0.47)		
	RX	22	11 (7.33)	11 (5.14)		
Vital status	Deceased	130	65 (42.48)	65 (29.82)	5.7932	0.016
	Living	241	88 (57.52)	153 (70.18)		

BACE1-AS, β-site APP-cleaving enzyme 1 antisense.

elevated *BACE1-AS* expression was an independent prognostic factor for both poor OS and RFS in patients with liver cancer. Overall, the data in the present study suggests *BACE1-AS* as a potential biomarker for diagnosis and prognostic classification of liver cancer.

BACE1-AS was found to be upregulated in liver cancer compared with healthy individuals, indicating its potential oncogenic role. Although AFP has been widely used as a marker for liver cancer, the low sensitivity and specificity has largely limited its value in cancer screening (3,22). Subsequently, the potential of *BACE1-AS* as a diagnostic marker in liver cancer was investigated. ROC analysis demonstrated both high sensitivity and specificity of *BACE1-AS* for diagnosing liver cancer. In order to test the clinical applicability of *BACE1-AS,* comparison with other well established/gold standard biomarkers is required. Although ultrasound combined with AFP testing represents currently the most popular strategy for liver cancer screening, such data are currently not available in the TCGA database. Nonetheless, the potential of *BACE1-AS* as a surrogate to AFP in liver cancer screening is worthy of further studies.

Table III. Univariate and multivariate analysis of overall survival in patients with liver cancer.

	Univariate analysis			Multivariate analysis		
Parameters	Hazard Ratio	95% CI (lower-upper)	P-value	Hazard Ratio	95% CI (lower-upper)	P-value
Age	1.02	0.7-1.48	0.926			
Sex	0.82	0.57-1.16	0.263			
Histological type	0.98	0.27-3.63	0.982			
Histologic grade	1.05	0.85-1.31	0.651			
Stage	1.38	1.15-1.65	0.001	0.85	0.69-1.06	0.151
T classification	1.65	1.38-1.98	0.000	1.83	1.46-2.3	0.000
N classification	0.71	0.5-1.03	0.071			
M classification	0.70	0.48-1.02	0.061			
Radiation therapy	0.52	0.26-1.03	0.061			
Residual tumor	1.42	1.12-1.79	0.004	1.43	1.12-1.83	0.004
BACE1-AS1	1.81	1.28-2.56	0.001	1.76	1.24-2.49	0.001

BACE1-AS, β-site APP-cleaving enzyme 1 antisense.

Table IV. Univariate and multivariate analysis of relapse free survival in patients with liver cancer.

	Univariate analysis			Multivariate analysis		
Parameters	Hazard Ratio	95% CI (lower-upper)	P-value	Hazard Ratio	95% CI (lower-upper)	P-value
Age	0.89	0.63-1.27	0.521			
Sex	0.98	0.69-1.4	0.919			
Histological type	2.03	0.66-6.29	0.218			
Histologic grade	0.98	0.8-1.21	0.873			
Stage	1.66	1.38-1.99	0.000	1.09	0.85-1.4	0.495
T classification	1.78	1.49-2.12	0.000	1.67	1.29-2.17	0.000
N classification	0.98	0.68-1.42	0.926			
M classification	1.19	0.8-1.78	0.394			
Radiation therapy	0.75	0.26-2.17	0.592			
Residual tumor	1.27	1.01-1.61	0.042	1.36	1.07-1.72	0.013
BACE1-AS1	1.65	1.18-2.31	0.003	1.58	1.13-2.22	0.008

BACE1-AS, β-site APP-cleaving enzyme 1 antisense.

Subgroup analysis revealed that *BACE1-AS* was differentially expressed in liver cancer among different categories. For example, *BACE1-AS* were highly associated with liver cancer histological grades, and *BACE1-AS* levels gradually increased as tumor grade was moving from G1 to G4, indicating that *BACE1-AS* may be an important factor in controlling tumor cell differentiation and the degrees of malignancy. Moreover, differential expression was also found in subgroups of different tumor staging and tumor size staging that constitutes the main parameter in tumor staging, suggesting its roles in tumor progression. The relative downregulation of *BACE1-AS* in stage IV and also T4 tumors may be a result of inadequate sample size in this particular subgroup. Thus, further analysis is urgently required. Interestingly, it was found that *BACE1-AS* levels varied among liver cancer of different histopathological groups, with a near significant overall trend P=0.063. *BACE1-AS* expression levels are significantly associated with histological types, when the continuous variable of *BACE1-AS* level is converted into binary value (P=0.043). This is important since the differential diagnosis between HCC and mixed HCC-CAA can be rather difficult in clinical settings through imaging (23,24). Traditional diagnostic method requires resection followed by thorough pathological examination. The implementation of *BACE1-AS* could be a potential marker in assisting differential diagnosis, which is crucial for later clinical decisions.

BACE1-AS is closely associated with clinical prognosis in liver cancer. However, subgroup analysis revealed

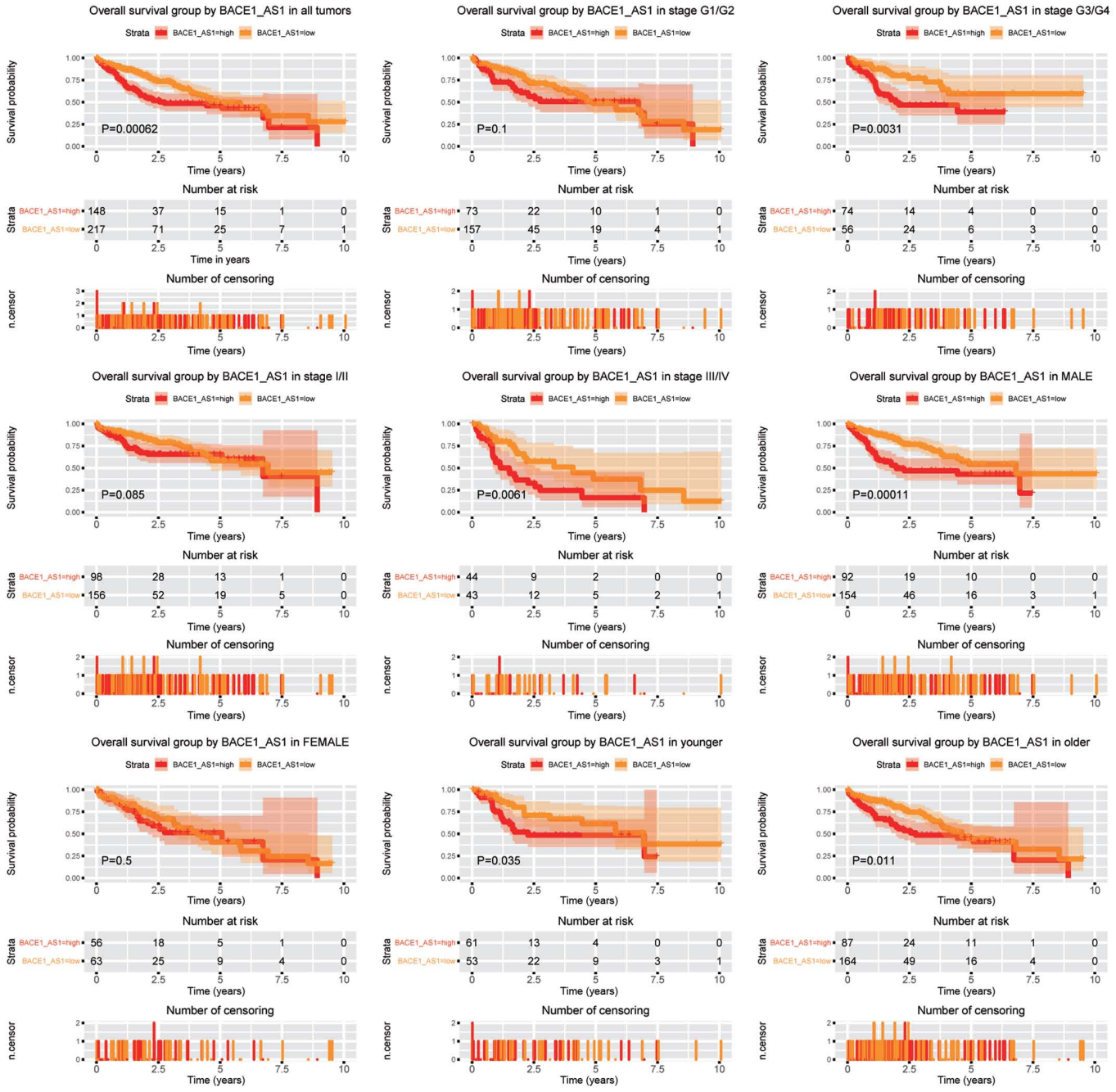

Figure 3. Overall survival outcomes according to BACE1-AS levels in different subgroups. Subgroups include tumor grades G1/G2, G3/G4, stage I/II, stage III/IV, males and females, younger and older patients. BACE1-AS, β-site APP-cleaving enzyme 1 antisense.

that *BACE1-AS* may not predict clinical outcome in some subgroups, such as in female patients. Overall, *BACE1-AS* is an unfavorable independent prognostic factor for both OS and RFS in liver cancer.

Mechanistically, *BACE1-AS* was first identified in AD as an antisense lncRNA to *BACE1*. The latter encodes a key β-secretase enzyme that is responsible for the formation of β-amyloid (Aβ) peptide, which is the central player in the pathogenesis of AD. It was experimentally confirmed that BACE1-AS could pair with the BACE1 mRNA and induce notable changes to the secondary or tertiary structures of the BACE1 mRNA, leading to increased BACE1 mRNA stability and translation in a positive feed-forward pathway. The present study explored whether this association also occurred in liver cancer. However, the results of the present study demonstrated no association between BACE1-AS and BACE mRNA expression (data not shown).

Furthermore, it is noteworthy that the results of the present study is in contrast to previous studies (11,12), in which *BACE1-AS* was demonstrated to function as a tumor suppressor. *BACE1-AS* was shown to be a novel target for anisomycin-mediated suppression of ovarian cancer stem cell proliferation and invasion (11). Elevated *BACE1-AS*, triggered by anisomycin treatment, leads to an increased accumulation of Aβ, which ultimately caused apoptosis of the ovarian cancer stem cells. Another study showed that *BACE1-AS* is downregulated in 5-fluorouracil-resistant colon cancer cells, suggesting its positive roles in chemosensitivity (12). The discrepancy could be generated from the type of

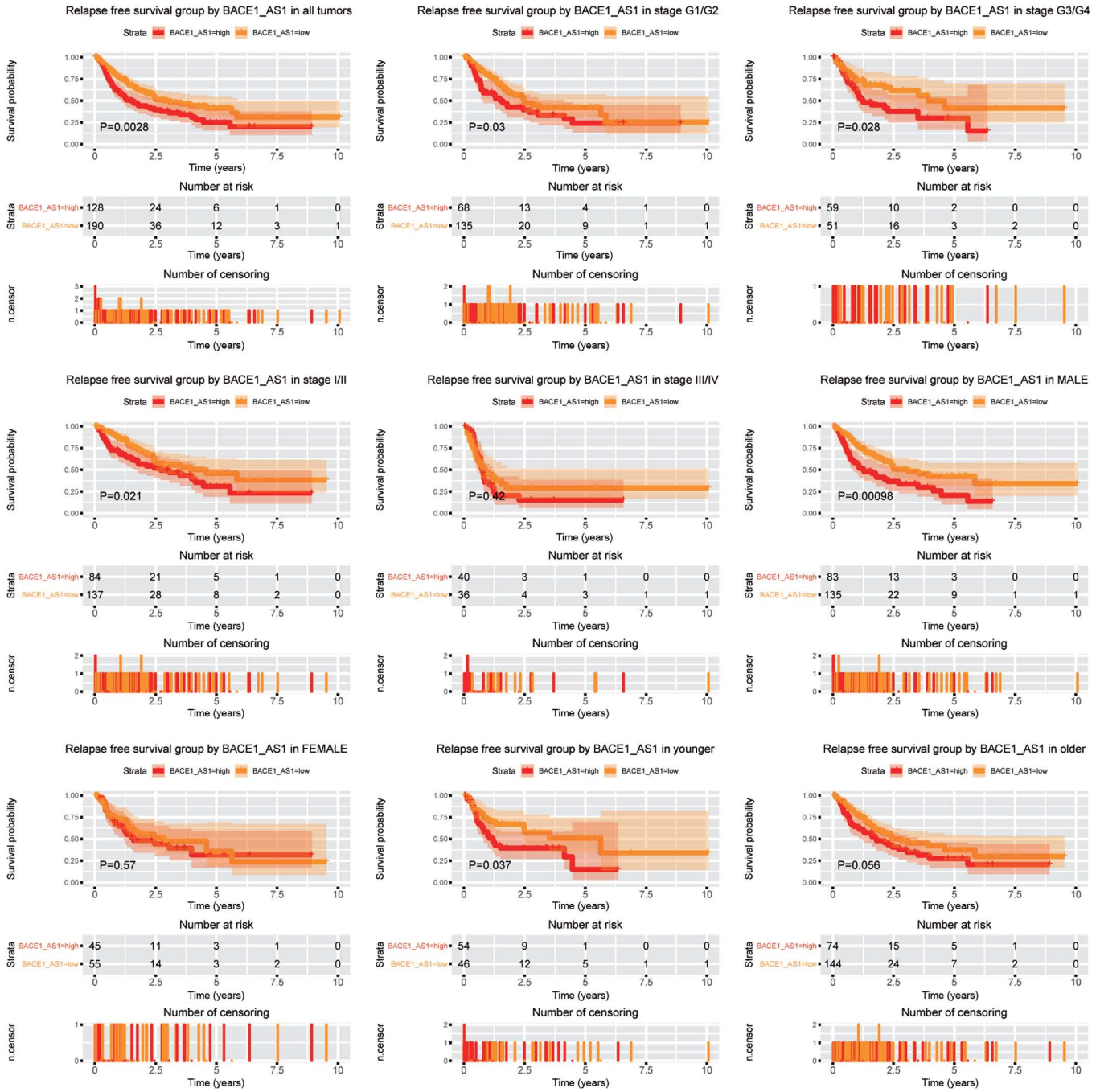

Figure 4. Relapse free survival outcomes according to different BACE1-AS levels in different subgroups. Subgroups include tumor grades G1/G2, G3/G4, stage I/II, stage III/IV, males and females, younger and older patients. BACE1-AS, β-site APP-cleaving enzyme 1 antisense.

studies. Previous studies mainly focused on *in vitro* experiments, whereas the present study was clinically centered. Moreover, the possibility that *BACE1-AS* might work in a context-dependent manner cannot be ruled out, the determination of which requires further studies both *in vitro* and *in vivo*.

It is worth noting that one possible limitation of the present study is the lack of validation by additional patient cohorts. Furthermore, other major prognostic factors such as liver function and liver-etiology were not included in the analysis, since such data are currently unavailable in the TCGA database. Nonetheless, the results of the present study raise the potential possibility of incorporating next generation sequencing data into clinical decision-making and paves way for further studies.

Overall, the present study is the first to demonstrate *BACE1-AS* as a potential diagnostic and prognostic biomarker in liver cancer. Further basic and clinical research is required in order to verify the results of the present study.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YN and YJ conceived and designed the study. YJ analyzed and interpreted the data with help from YL and YX. YN drafted the manuscript. WL analyzed and interpreted data and revise the manuscript for important intellectual content. All authors have read and approved of the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patients' consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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