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DATABASE ANALYSIS

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Small Nucleolar RNAs (snoRNAs)-Based Risk Score Classifier Predicts Overall Survival in Bladder Carcinoma

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Background: Bladder carcinoma (BLCA) is a leading cause of cancer-related deaths worldwide. The aim of this work was to develop an accurate stratification in predicting the prognosis and directing the treatment of BLCA patients based on small nucleolar RNAs (snoRNAs).

Material/Methods: Expression profiles of snoRNAs were downloaded from the SNORic database. The expression profiles and clinical outcomes of BLCA patients were analyzed. Survival-associated snoRNAs were identified and used to develop a novel risk score classifier. Genes in the whole genome that were significantly correlated with the included prognostic snoRNAs were used for functional enrichment analysis.

Results: The results showed that age, American Joint Committee on Cancer (AJCC) stage, and tumor status were significantly correlated with overall survival (OS) of BLCA patients. We selected 12 survival-associated snoRNAs to build a prognostic signature. Patients were separated into high- and low-risk groups based on the median value of the risk score. Patients in the high-risk group and low-risk group have distinct clinical outcomes. The AJCC TNM stage showed moderate utility as a prognostic indicator for clinical outcome prediction. Then, clinical parameters and risk scores were entered in multivariate Cox analysis. Notably, the prognostic signature remained an independent significant prognostic risk factor. The pathway analysis suggested that these genes were enriched in several types of cancer and "Focal adhesion" pathways.

Conclusions: The prognostic signature defined by expression profiles of 12 survival-associated snoRNAs appears to be an excellent predictor of the clinical outcome of BLCA patients.

MeSH Keywords: **Risk Factors • RNA, Small Untranslated • Survival Rate • Urinary Bladder Neoplasms**

Abbreviations: **BLCA** – bladder carcinoma; **snoRNAs** – small nucleolar RNAs; **AJCC** – American Joint Committee on Cancer; **TCGA** – The Cancer Genome Atlas; **ROC** – receiver operating characteristic; **OS** – overall survival; **KEGG** – Kyoto Encyclopedia of Genes and Genomes; **PPAR** – peroxisome proliferator-activated receptor; **ECM** – extracellular matrix

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Background

Bladder cancer is a heterogeneous malignancy that is responsible for an estimated 81 400 new cases and 17 980 deaths in the United states in 2020 [1]. Although progress has been made in surgical resection technology and neoadjuvant chemotherapy, survival after surgical resection differs considerably among BLCA patients [2–4]. The predictive accuracy of the current clinical staging system is insufficient for this medically precise era and for the display of the molecular characteristics of BLCA. Hence, cancer researchers have been interested in developing an accurate stratification method that can predict the prognosis and direct treatment of BLCA patients to improve their survival [5–10]. It is also imperative to determine the biological characteristics of BLCA.

Genome researchers have long regarded the non-coding RNAs of the human genome as 'junk' DNA [11]. Specifically, the rapid development of high-throughput technology helped scientists to identify a large number of non-coding RNAs, which account for almost 60% of the transcriptional output in human cells [12–16]. This huge number of non-coding RNAs makes it difficult to dismiss them as "junk". Hence, recent extensive research has identified that non-coding RNAs, especially long non-coding RNAs and micro RNAs, are directly linked to the tumorigenesis and development of cancers [17,18]. Notably, small nucleolar RNA (snoRNAs), a class of small (60–300 nucleotides) non-coding RNAs, has been well documented in rRNA biogenesis. Recently, however, some studies have highlighted that snoRNAs are involved in cancer development and progression [19]. Because these studies dismissed the non-coding RNAs of the human genome as 'junk' DNA, the relationships between snoRNAs and BLCA have not been well characterized.

The Cancer Genome Atlas (TCGA) consortium and other public genomic datasets offer high-throughput data for the subclassification of BLCA, as well as predicting diagnosis and prognosis [20–22]. Gong et al. developed a database of snoRNA in cancers to systematically quantify and deposit snoRNA expression profiles in more than 10 000 samples across 31 cancer types based on TCGA, including BLCA [23]. This work greatly promoted the development of snoRNAs analysis in cancers. However, very few other studies have explored the role of snoRNAs in BLCA. Furthermore, no investigative reports on models that predict the survival status of BLCA based on snoRNAs are available.

Based on TCGA data portal, we analyzed the expression profiles and clinical outcomes of BLCA patients. Survival-associated snoRNAs were identified and submitted to develop a novel 12-snoRNAs-based risk score classifier. To leverage the complementary value of molecular and clinical parameters, clinical factors were integrated to build a nomogram, which allowed improved prediction of BLCA patient survival.

Material and Methods

Data Acquisition

Expression profiles of snoRNAs were downloaded from the SNORic database [23]. Corresponding clinical data of BLCA patients were acquired from the TCGA database (*https://cancergenome.nih.gov/*).

Survival analysis

To generate a prognostic classifier to predict the outcome of BLCA, we used univariate Cox analysis to explore the relationships between snoRNAs expression levels and the overall survival (OS) of BLCA patients. Patients with OS less than 90 days were removed to obtain more accurate results [24]. Then, multivariate Cox regression analysis was conducted to screen out independent prognostic factors. We calculated a signature involving the single prognostic parameters achieved from the previous step to assess the OS risk based on the individual expression of the prognostic snoRNAs, weighted by the regression coefficient. The data were then divided into high- or low-risk groups by using the median risk score as the threshold value. To leverage the complementary value of the snoRNAs and several indispensable clinical parameters, including age, sex, tumor T stage, tumor N stage, tumor M stage, and histological grade, we integrated them by using a nomogram graph.

Functional characterization of the snoRNAs-based risk score

To further gain a biological understanding of the snoRNAs-based risk score, we collected genes in the whole genome which were significantly correlated with the included prognostic snoRNAs that were obtained from SNORic. Significantly correlated genes were identified with the |Spearman coefficient| ≥0.4 and FDR <0.05. These significantly correlated genes were submitted to a "clusterProfiler" package in R software for gene functional enrichment analysis. The background of the gene list was measured by "Homo sapiens." Statistically significant terms were identified when the p-value and the q-value were both less than 0.05.

Statistical analysis

All statistical analyses were performed using SPSS (version 22.0, SPSS Inc., Chicago, IL, USA) and R (version 3.3.1; *https://www.r-project.org/*). snoRNAs expression values were converted to log2 (RMPK+1) and the average expression value across all samples less than 1 were identified as undetected snoRNAs. The difference for snoRNAs with OS was evaluated using the log-rank test. Univariate survival Cox analysis was performed by using "survival" packages of R software. Time-dependent

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Table 1. Clinical information of included BLCA patients.

	No.	Median time (days)	Event	Censored
Age				
≥65	226	545	116	110
<65	140	586	43	97
Gender				
Male	271	565	112	259
Female	95	560	47	48
AJCC stage				
Stage III–IV	245	544	127	118
Stage I–II	119	638	31	88
NA	2	779.5	1	1
AJCC T-stage				
T3–T4	177	544	91	86
T1–T2	183	588	67	116
TX	6	692.5	1	5
AJCC N-stage				
N1–N3	119	536	75	44
N0	211	578	67	144
NX	36	613.5	17	19
AJCC M-stage				
M1	9	460	6	3
M0	174	578	64	110
MX	183	565	89	94
Histological grade				
High Grade	345	577	157	188
Low Grade	18	383.5	2	16
NA	3	578	0	3

(receiver operating characteristic) ROC curve and corresponding area under the curve (AUC) values, which could assess the performance of prognostic signatures, were calculated by using the "survivalROC" package. The nomogram graph was derived by using the "RMS" package. Statistical significance was defined as $P<0.05$, unless specified otherwise.

Results

Identification of survival-associated snoRNAs

Expression profiles of 412 snoRNAs were acquired from the SNORic database. Finally, a total of 366 BLCA patients with OS more than 90 days were included in the present study. Table 1 shows the clinical information of the patients with BLCA from TCGA. Univariate Cox hazard analyses were performed to assess the relationship between clinical parameters and clinical outcomes of the BLCA patients. Age (HR=1.792, 95% CI: 1.262–2.543; $P=0.001$) and AJCC stage (HR=2.161, 95% CI: 1.459–3.202; $P<0.001$) were significantly correlated with OS of BLCA patients. However, no significant correlations were observed between OS and sex or histological grade. All 368 snoRNAs were submitted to a univariate Cox analysis and 58 snoRNAs were identified as survival-associated snoRNAs (Figure 1, Table 2).

Construction of snoRNAs-based prognostic signature

Significant factors from univariate selection were kept in the multivariate analysis by using backward selection (Table 3). A multivariate Cox regression analysis was used to build a prognostic signature that selected 12 out of the 58 snoRNAs. Kaplan-Meier (K-M) curves were used to display the prognostic

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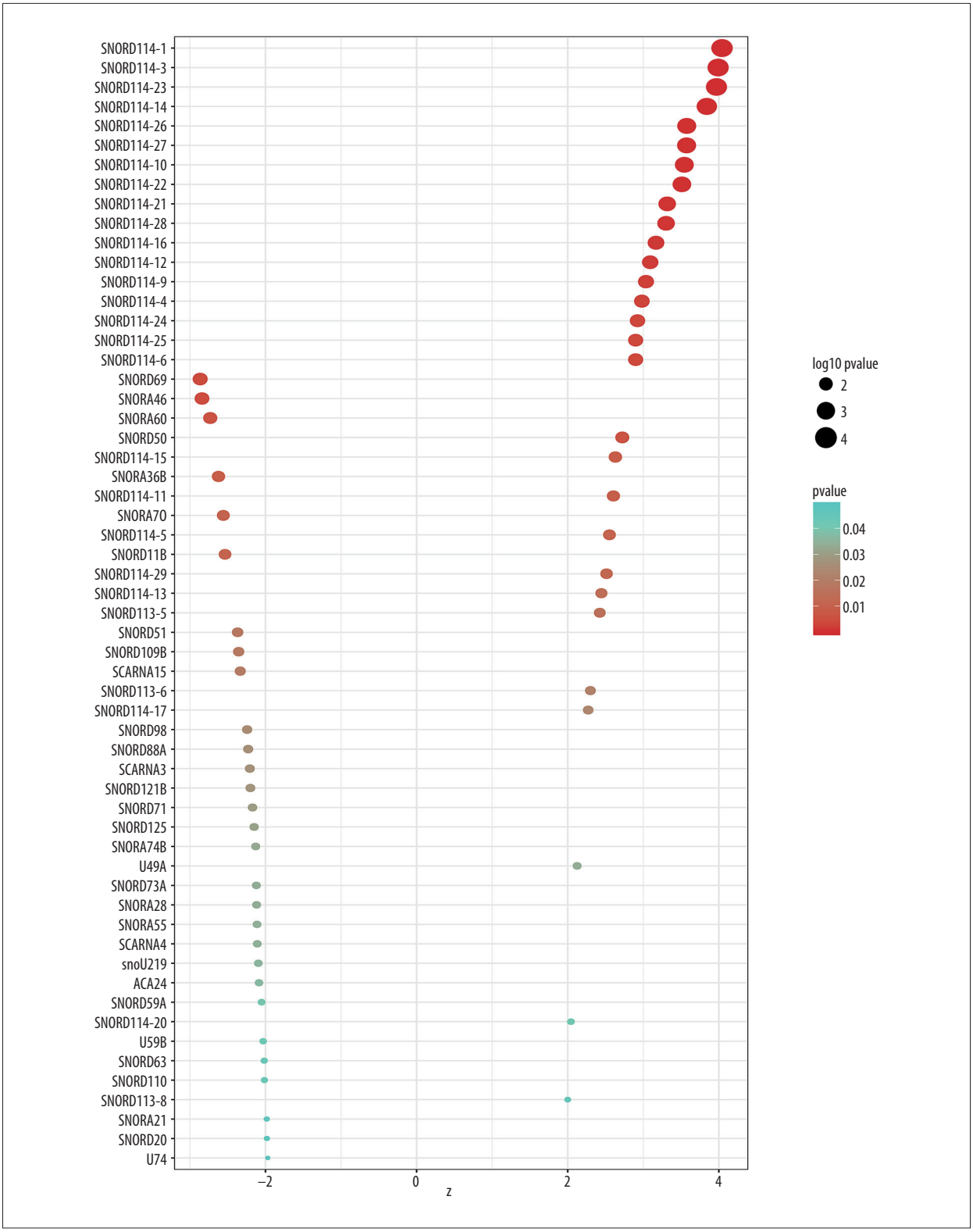

Figure 1. Survival-associated snoRNAs in BLCA. X-axis represents the Z-score of the snoRNAs in univariate Cox analysis and Y-axis represents survival-associated snoRNAs. Thresholds are p<0.05 and |Z-score| >1.8

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Table 2. General characteristics of prognosis-related snoRNAs in BLCA.

SnoRNA	Ensemble id	Chromosome location	HR	Z-score	P-value
SNORD114-1	ENSG00000199575	chr14_101416169_101416241	1.172157633	4.042243541	5.29E-05
SNORD114-3	ENSG00000201839	chr14_101419685_101419760	1.244460803	3.989900024	6.61E-05
SNORD114-23	ENSG00000200406	chr14_101450212_101450284	1.200432991	3.967968648	7.25E-05
SNORD114-14	ENSG00000199593	chr14_101438439_101438514	1.225257693	3.841568643	0.000122251
SNORD114-26	ENSG00000200413	chr14_101453382_101453454	1.177498875	3.574101976	0.000351432
SNORD114-27	ENSG00000200636	chr14_101454497_101454567	1.204509378	3.572399323	0.000353725
SNORD114-10	ENSG00000200279	chr14_101433388_101433460	1.195695701	3.544136258	0.000393902
SNORD114-22	ENSG00000202293	chr14_101449262_101449334	1.184810744	3.510696121	0.000446935
SNORD114-21	ENSG00000272344	chr14_101448311_101448383	1.166642474	3.314913224	0.000916716
SNORD114-28	ENSG00000200480	chr14_101455466_101455538	1.181825508	3.300466355	0.000965243
SNORD114-16	ENSG00000199914	chr14_101439931_101440001	1.141680142	3.167935272	0.001535257
SNORD114-12	ENSG00000202270	chr14_101435284_101435359	1.167420569	3.09099896	0.001994843
SNORD114-9	ENSG00000201240	chr14_101432365_101432437	1.177886029	3.036379423	0.002394379
SNORD114-4	ENSG00000200832	chr14_101420710_101420785	1.196448159	2.980747353	0.002875459
SNORD114-24	ENSG00000201899	chr14_101451113_101451185	1.145736023	2.922467683	0.003472697
SNORD114-25	ENSG00000200612	chr14_101452393_101452465	1.168691075	2.897966743	0.003755904
SNORD114-6	ENSG00000201263	chr14_101423502_101423574	1.131317371	2.89790986	0.003756585
SNORD69	ENSG00000212452	chr3_52726751_52726828	0.829923328	−2.863363869	0.004191689
SNORA46	ENSG00000207493	chr16_58582402_58582537	0.817200622	−2.840019864	0.004511072
SNORA60	ENSG00000199266	chr20_37078011_37078147	0.831925367	−2.731873135	0.006297539
SNORD50	ENSG00000202335	chr12_110934157_110934226	1.092412377	2.722243241	0.00648404
SNORD114-15	ENSG00000201557	chr14_101439006_101439078	1.125731167	2.630556393	0.008524523
SNORA36B	ENSG00000222370	chr1_220373887_220374018	0.7897178	−2.621196299	0.008762179
SNORD114-11	ENSG00000200608	chr14_101434447_101434522	1.109513238	2.604648921	0.009196844
SNORA70	ENSG00000206661	chr8_4985801_4985934	0.845867576	−2.55813685	0.010523467
SNORD114-5	ENSG00000199798	chr14_101421706_101421776	1.130225789	2.552064338	0.010708674
SNORD11B	ENSG00000271852	chr2_203156054_203156144	0.874855705	−2.535644644	0.011224057
SNORD114-29	ENSG00000201689	chr14_101456427_101456497	1.119666412	2.513808912	0.011943513
SNORD114-13	ENSG00000201247	chr14_101436215_101436289	1.127416664	2.445552573	0.014463036
SNORD113-5	ENSG00000272474	chr14_101404523_101404601	1.139249056	2.423952021	0.015352634
SNORD51	ENSG00000207047	chr2_207026602_207026681	0.837567821	−2.368768099	0.01784744
SNORD109B	ENSG00000239169	chr15_25523489_25523556	0.866810843	−2.354682907	0.018538518
SCARNA15	ENSG00000252193	chr20_41933195_41933319	0.867935397	−2.333853912	0.019603373
SNORD113-6	ENSG00000200215	chr14_101405892_101405968	1.119284272	2.300058669	0.021444896

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Table 2 continued. General characteristics of prognosis-related snoRNAs in BLCA.

SnoRNA	Ensemble id	Chromosome location	HR	Z-score	P-value
SNORD114-17	ENSG00000201569	chr14_101441142_101441217	1.146335348	2.270526772	0.023175641
SNORD98	ENSG00000221182	chr10_70514928_70514995	0.848508292	–2.242967391	0.024898917
SNORD88A	ENSG00000221241	chr19_51302695_51302792	0.870328188	–2.22784215	0.025891043
SCARNA3	ENSG00000252906	chr1_175937532_175937676	0.842498005	–2.207789545	0.027258947
SNORD121B	ENSG00000238300	chr9_33934294_33934374	0.881577227	–2.199487508	0.027843276
SNORD71	ENSG00000223224	chr16_71792304_71792390	0.828580666	–2.172435589	0.029822823
SNORD125	ENSG00000239127	chr22_29729151_29729247	0.866129363	–2.151538303	0.031433736
SNORA74B	ENSG00000212402	chr5_172447728_172447932	0.836039977	–2.129319367	0.033227847
U49A	NA	chr17_16343349_16343420	1.130835565	2.123119267	0.033743859
SNORD73A	ENSG00000208797	chr4_152024978_152025043	0.89226166	–2.119595454	0.034040176
SNORA28	ENSG00000272533	chr14_103804185_103804311	0.865292844	–2.117394539	0.034226377
SNORA55	ENSG00000201457	chr1_40033045_40033182	0.872055098	–2.112023658	0.03468442
SCARNA4	ENSG00000252808	chr1_155895748_155895877	0.89074157	–2.109787332	0.034876678
snoU219	ENSG00000201592	chrX_20154424_20154503	0.876558632	–2.094904314	0.036179499
ACA24	NA	chr4_119200344_119200475	0.910089166	–2.086331357	0.036948618
SNORD59A	ENSG00000207031	chr12_57038810_57038885	0.862298053	–2.051283898	0.040239308
SNORD114-20	ENSG00000202048	chr14_101447340_101447412	1.090210857	2.041361273	0.041214931
U59B	NA	chr12_57037463_57037538	0.846756387	–2.031920052	0.04216175
SNORD63	ENSG00000206989	chr5_137896731_137896799	0.85784948	–2.017395329	0.043654274
SNORD110	ENSG00000221116	chr20_2634857_2634932	0.86206084	–2.014186617	0.043989944
SNORD113-8	ENSG00000200367	chr14_101409787_101409861	1.115240661	1.999711306	0.045531447
SNORA21	ENSG00000199293	chr17_37009115_37009248	0.897130856	–1.982249792	0.04745129
SNORD20	ENSG00000207280	chr2_232321154_232321234	0.861938927	–1.981243636	0.047563958
U74	NA	chr1_173836811_173836883	0.87740203	–1.969834633	0.048857326

value of each snoRNA (Figure 2). We then derived a prognostic signature for each patient based on the individual expression levels of the 12 survival-associated snoRNAs multiplied by their coefficients in the multivariate Cox analysis: prognostic signature=(SNORD114–11 * (–0.168)+SNORD114–14 * (0.201)–SNORD114–15 * (0.229)+SNORD114–9 * (0.543)–SNORA55 * (0.198)–SNORA60 * (0.192)–SNORD88A * (0.167)–SNORD69 * (0.314)–SNORD20 * (0.226)+U49A * (0.432)–SNORD51 * (0.335)+U74 * (0.514) (Figure 3). Patients were separated into high- and low-risk groups by the median value of the prognostic signature. K-M survival plots indicated that patients in the high- and low-risk groups had distinct clinical outcomes (HR=2.500, 95% CI: 1.828–3.420, *P*<0.001; Figure 4A). The AUC value of the ROC curve was 0.719. The threshold was 2000 days (Figure 4B). The AJCC TNM stage appears to be a moderate prognostic indicator for clinical outcome predicting (HR=2.155, 95% CI: 1.553–2.993, *P*<0.001; Figure 4C). The AUC value of the TNM stage was 0.636 (Figure 4D). We integrated the clinical factors and risk score to create a composite nomogram based on the results of the multivariate Cox regression analyses to predict 1-year, 2-year, and 3-year OS. (Figure 5). We then submitted clinical parameters and risk scores in multivariate Cox analysis. Notably, the prognostic signature remained an independent significant prognostic risk factor (HR=3.300, 95% CI: 2.203–4.943, *P*<0.001) (Table 4).

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Table 3. The results of multivariate analysis selection.

snoRNA	β	SE	Wald	Sig.	Exp(B)	95.0% CI for Exp(B)
SNORD114-11	–0.168	0.072	5.541	0.019	0.845	0.735–0.972
SNORD114-14	0.201	0.099	4.141	0.042	1.223	1.007–1.485
SNORD114-15	–0.229	0.099	5.421	0.02	0.795	0.655–0.964
SNORD114-9	0.543	0.132	16.928	<0.001	1.72	1.329–2.228
SNORA55	–0.198	0.076	6.739	0.009	0.82	0.707–0.953
SNORA60	–0.192	0.084	5.212	0.022	0.825	0.699–0.973
SNORD88A	–0.167	0.081	4.211	0.04	0.846	0.722–0.993
SNORD69	–0.314	0.116	7.349	0.007	0.73	0.582–0.917
SNORD20	–0.226	0.128	3.153	0.076	0.797	0.621–1.024
U49A	0.432	0.084	26.213	<0.001	1.541	1.306–1.818
SNORD51	–0.335	0.123	7.441	0.006	0.715	0.562–0.91
U74	0.514	0.148	12.032	0.001	1.672	1.250–2.235

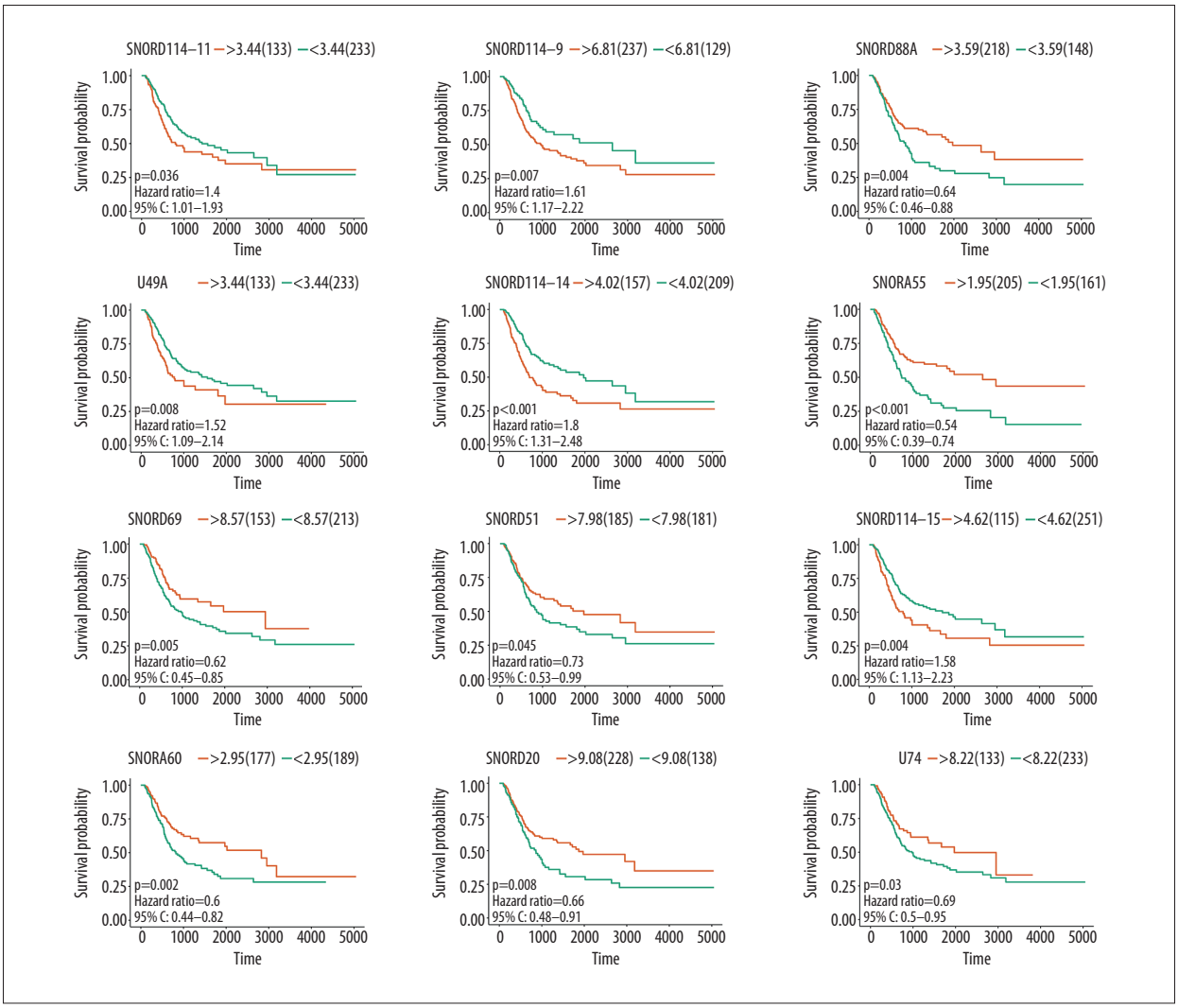

Figure 2. K-M plots prognostic snoRNAs included in prognostic signature.

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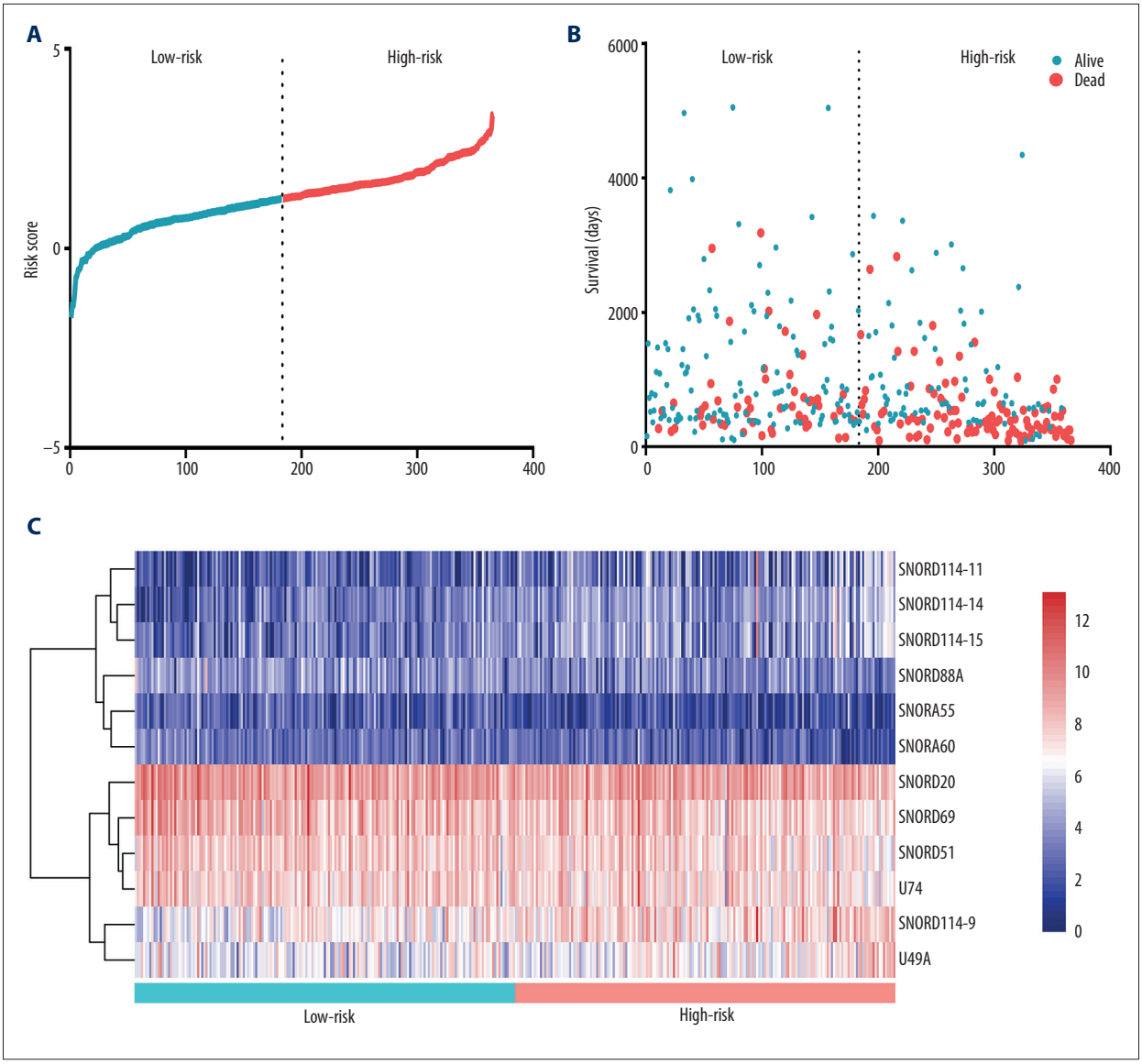

Figure 3. Prognostic signature constructed based on 12 snoRNAs. (**A**) The risk score assigned to each patient; (**B**) Survival status of BLCA patients in high- and low-risk group; and (**C**) The expression pattern of included snoRNAs.

Molecular function of prognostic signature

Genes that were significantly correlated with 12 survival-associated snoRNAs were obtained from SNORic based on Spearman correlation analysis. The network was conducted to display the relationships between snoRNAs and genes (Figure 6). Red lines indicate positive correlation relationships while blue lines indicate negative correlation relationships. SnoRNAs-related genes were further submitted to gene functional enrichment analysis. For the biological process, the extracellular structure organization, the extracellular matrix organization, and skeletal system development were the commonly enriched categories (Figure 7A). For the cellular component ontology, the enriched categories were correlated with proteinaceous extracellular matrix, endoplasmic reticulum lumen, and contractile fiber (Figure 7B). With regards to the molecular function, the snoRNA-related genes mainly showed enrichment in cell adhesion molecule binding, actin binding, and sulfur compound binding (Figure 7C). The disease ontology suggested that these genes were enriched in several types of cancer (Figure 8A). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed significant pathways with these genes (Figure 8B). "Focal adhesion" was the most significant of the enriched terms.

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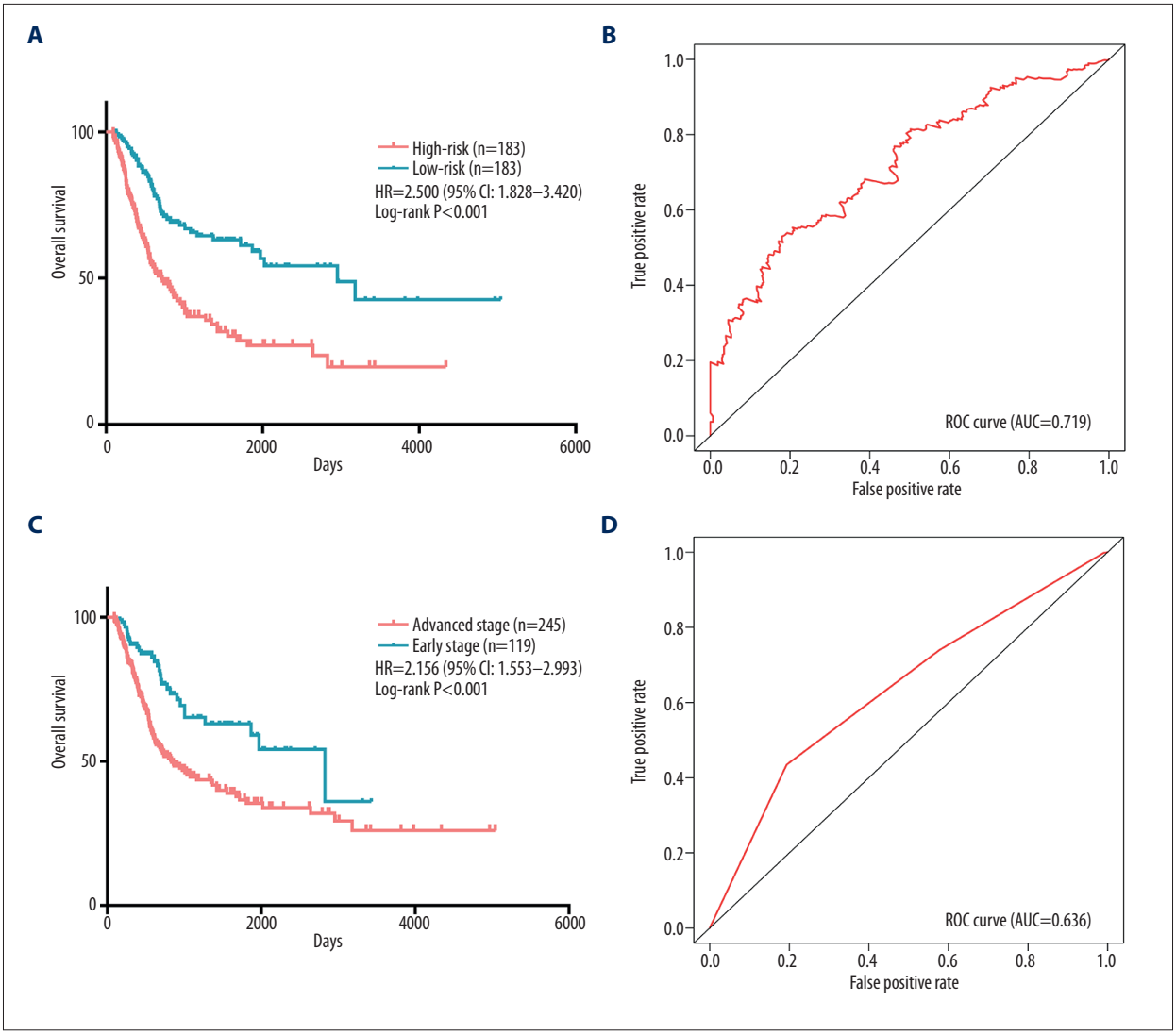

Figure 4. Comparation of snoRNA-based prognostic signature and AJCC_TNM stage in predicting the clinical outcome of BLCA patients. (**A**) K-M survival plots indicated that patients in the high-risk group tended to have poor clinical outcomes; (**B**) ROC curves with AUCs of prognostic predictors built by snoRNAs in BLCA; (**C**) K-M survival plots indicated that patients in advanced stage tended to have poor clinical outcomes; and (**D**) ROC curves with AUCs of AJCC_TNM stage.

Discussion

Here, we performed a systematic analysis of the snoRNAs and identified a risk score based on the expression profiles of 12 survival-associated snoRNAs in BLCA patients based on 366 clinical cases. Our study resulted in the following: (1) the identification of 58 prognostic relevant snoRNAs; (2) the development of a twelve-snoRNAs-based risk score classifier that predicts OS in BLCA; (3) a pathway analyses that revealed the molecular characteristics of the risk score; and (4) the construction of a nomogram to leverage the complementary value of molecular and clinical factors.

We first identified several snoRNAs that were correlated with the clinical outcomes of BLCA. Given the stable nature of snoRNAs in the human body, these snoRNAs have inherent advantages for use as molecular biomarkers [25–27]. Regrettably, few studies have assessed snoRNAs as diagnostic and prognostic tools for BLCA. This is mainly due to the conventional prejudice that snoRNAs mainly function to modify, mature, and stabilize rRNAs [28]. Furthermore, high-throughput DNA sequencing techniques help to identify cancer-specific snoRNAs [29]. Hence, our group comprehensively analyzed the clinical significance and potential molecular characteristics of snoRNAs to identify the diagnostic and prognostic biomarkers in BLCA.

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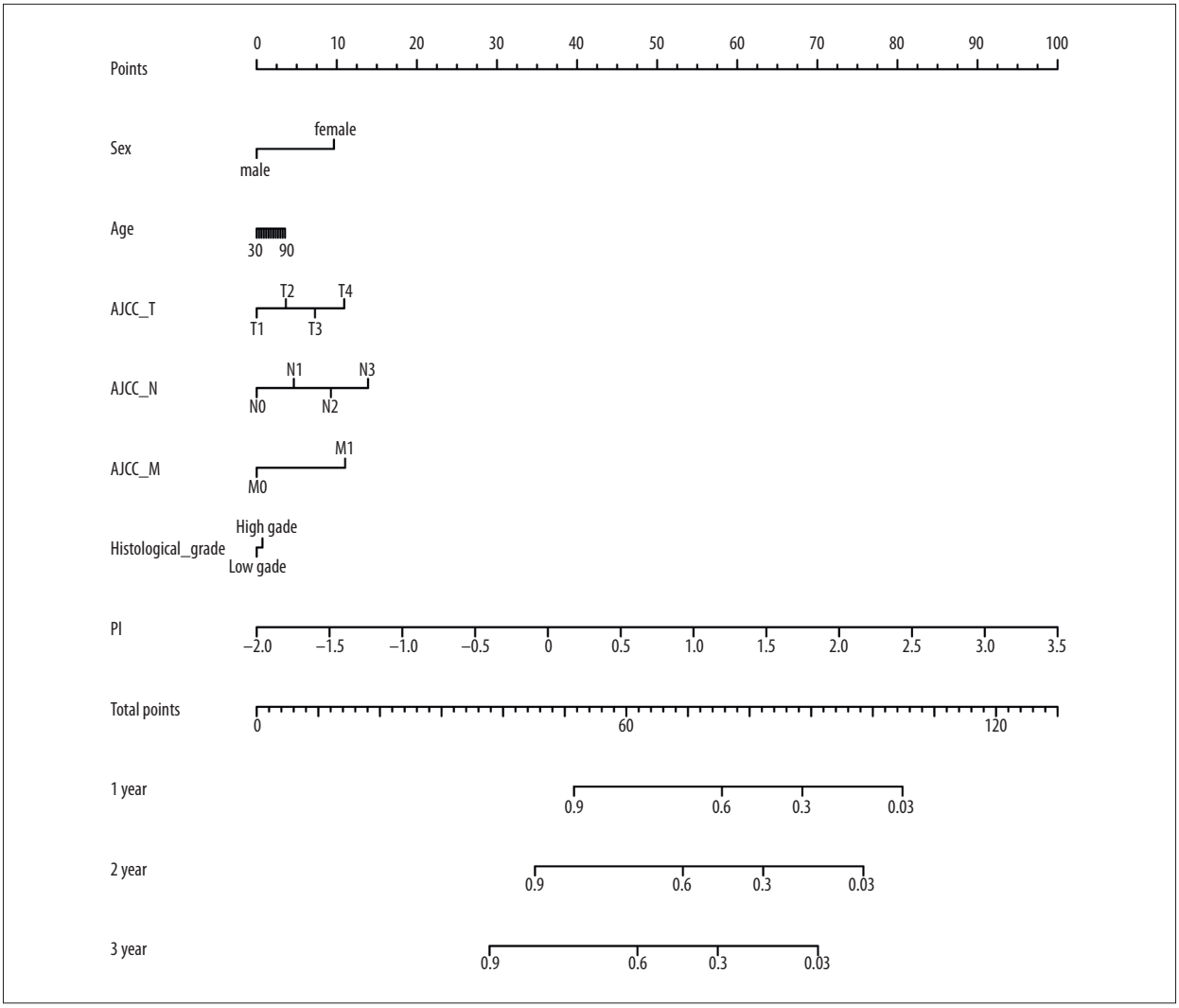

Figure 5. Nomogram of BLCA patients.

Table 4. Multivariate Cox analysis of OS in BLCA patients of TCGA.

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	1.031 (1.014–1.047)	<0.001	1.004 (0.975–1.033)	0.793
Gender	0.827 (0.588–1.162)	0.274	0.532 (0.297–0.952)	0.033
AJCC_T stage	1.651 (1.293–2.108)	<0.001	1.271 (0.793–2.037)	0.319
AJCC_N stage	1.588 (1.339–1.883)	<0.001	1.354 (0.975–1.880)	0.070
AJCC_M stage	2.930 (1.258–6.825)	0.013	2.045 (0.637–6.566)	0.229
Histologic grade	2.557 (0.631–10.351)	0.188	0.955 (0.121–7.558)	0.965
SnoRNAs-based risk score	2.718 (2.131–3.467)	<0.001	3.300 (2.203–4.943)	<0.001

Age, AJCC_T stage, AJCC_N stage, snoRNAs-based risk score was coded as continuous variables. Specifically, AJCC_T stage was coded as T1=1, T2=2, T3=3, T4=4. AJCC_N stage was coded as N0=0, N1=1, N2=2, N3=3. The risk factors of gender, AJCC_M stage, subtype and histologic grade are male, metastasis and high Grade.

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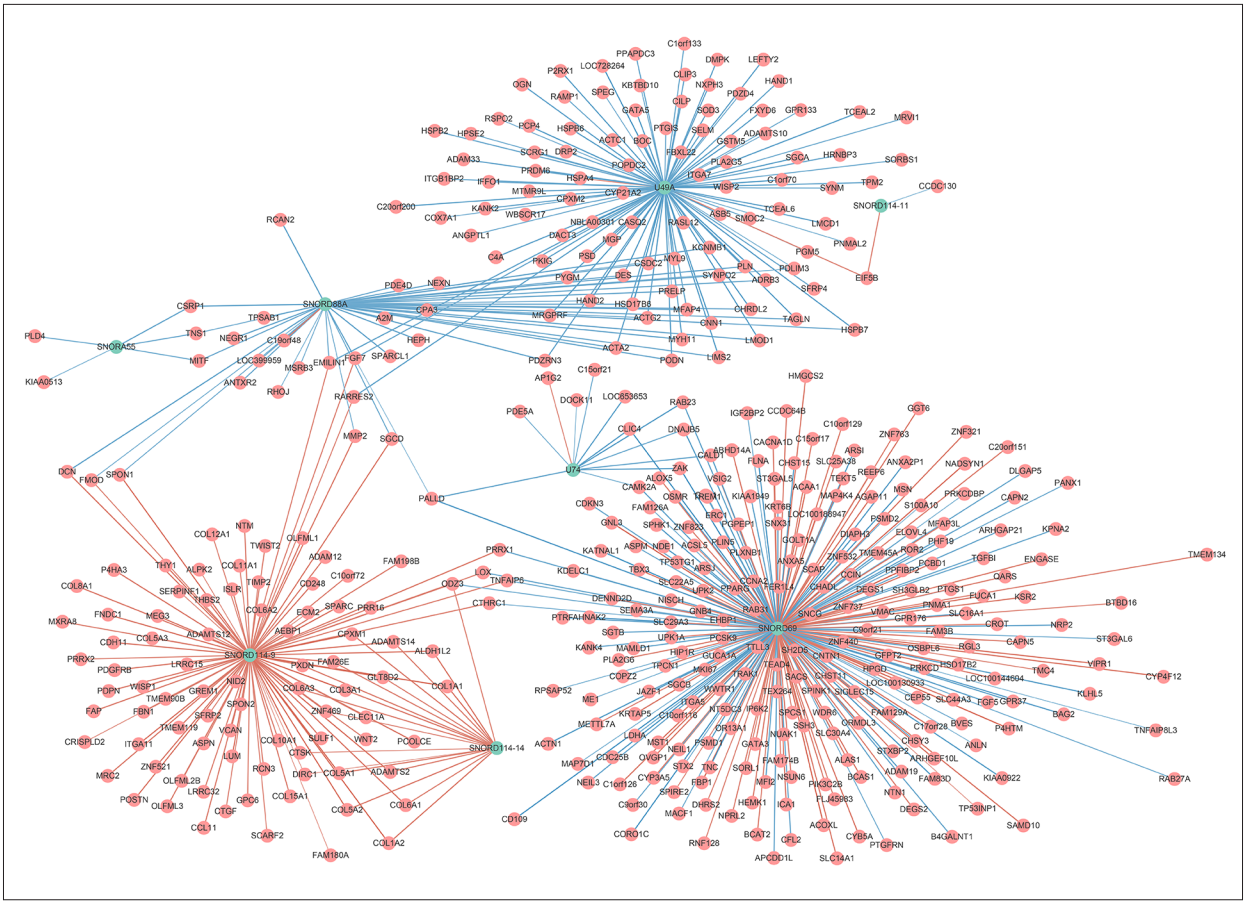

Figure 6. Correlation network of included snoRNAs and mRNAs. Expression of survival-associated snoRNAs (green dots) were positively (red line)/negatively (blue line) correlated with the expression level of mRNAs (red dots).

An important goal of the present study was the construction of risk scores based on the expression pattern of snoRNAs to create a risk stratification model for the practice of precision medicine. Previously, studies involved several molecules, including mRNA expression, copy number variation, DNA methylation, lncRNAs, and miRNAs. For example, Liu et al. proposed a clinical multidimensional transcriptome signature for survival predictions of patients with BLCA [30]. Aberrant DNA methylation can provide reliable biomarkers in the prediction of clinical outcomes of common urological cancers [31]. Several studies have explored the prognostic value of non-coding RNAs in developing prognostic signatures for BLCA [32,33]. We constructed the risk score model with the expectation that it would be applicable to clinical management from the perspectives of snoRNAs. On the one hand, studies that refer to the clinical significance of snoRNAs are limited. The present study provides novel insights into the clinical value and molecular mechanisms of snoRNAs in BLCA. On the other hand, we optimized the prognostic model and considered both molecular and clinical features. The snoRNAs possess the possibility and feasibility as biomarkers and therapeutic targets in clinical use. Aberrant snoRNAs expression was found in many cancers, and the expression level was correlated with diagnosis, classification of subtypes, and patient survival. Moreover, snoRNAs were stably expressed and detectable in body fluids, including blood plasma, serum, and urine of cancer patients, indicating that snoRNAs have the potential to serve as biomarkers in clinical use. Urine testing is very important for early diagnosis of BCLA. Since snoRNAs are stably expressed and detectable in urine, snoRNAs detection in urine might be useful for BLCA diagnosis and prognosis prediction [34–36].

It is interesting to explore the biological characteristics reflected by the risk score. Nowadays, the cellular regulatory roles of snoRNAs in cancers are widely understood. Hence, we selected snoRNAs-related genes to explore the molecular characteristics of snoRNAs in BLCA. We found that prognostic snoRNAs are mainly involved in several signal transduction pathways, such as focal adhesion, extracellular matrix (ECM)-receptor interaction, and peroxisome proliferator-activated receptor (PPAR) signaling pathway. In these findings, the risk score could reflect the cell-cell interaction status of BLCA. Notably, focal adhesion was the most significant KEGG pathway in the pathway functional enrichment analysis. In biological activity, focal adhesion

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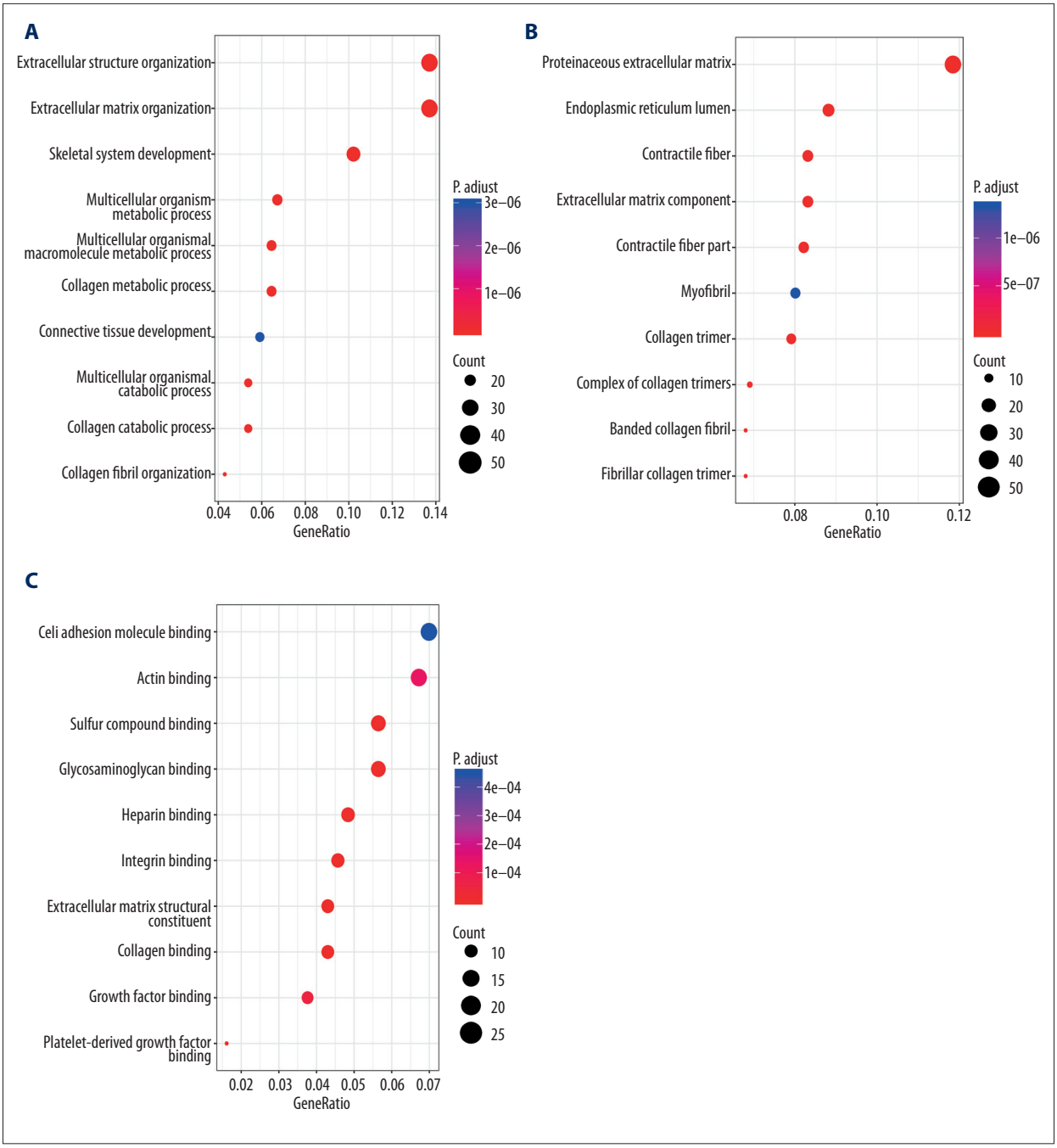

Figure 7. Gene ontology of prognostic signature-related genes. (**A**) Biological process; (**B**) Cellular component; (**C**) Molecular function.

is a sub-cellular regulatory structure that mediates mechanical force and regulatory signals transmitted between the ECM and an interacting cell [37]. The focal adhesion pathway interacts closely with other indispensable oncogenic pathways and is actively involved in the progression of cancers [38,39]. Interestingly, inhibitors focused on the focal adhesion pathway could be effective anti-tumor targets [40]. The biological characteristics of the risk score are correlated with cell-cell interactions, which indicated that these snoRNAs actively participate in the progression and/or metastasis of BLCA, although the results need to be further explored.

Conclusions

In summary, the development of a prognostic signature, as defined by expression profiles of 12 survival-associated snoRNAs, could be an excellent predictor of the clinical outcome

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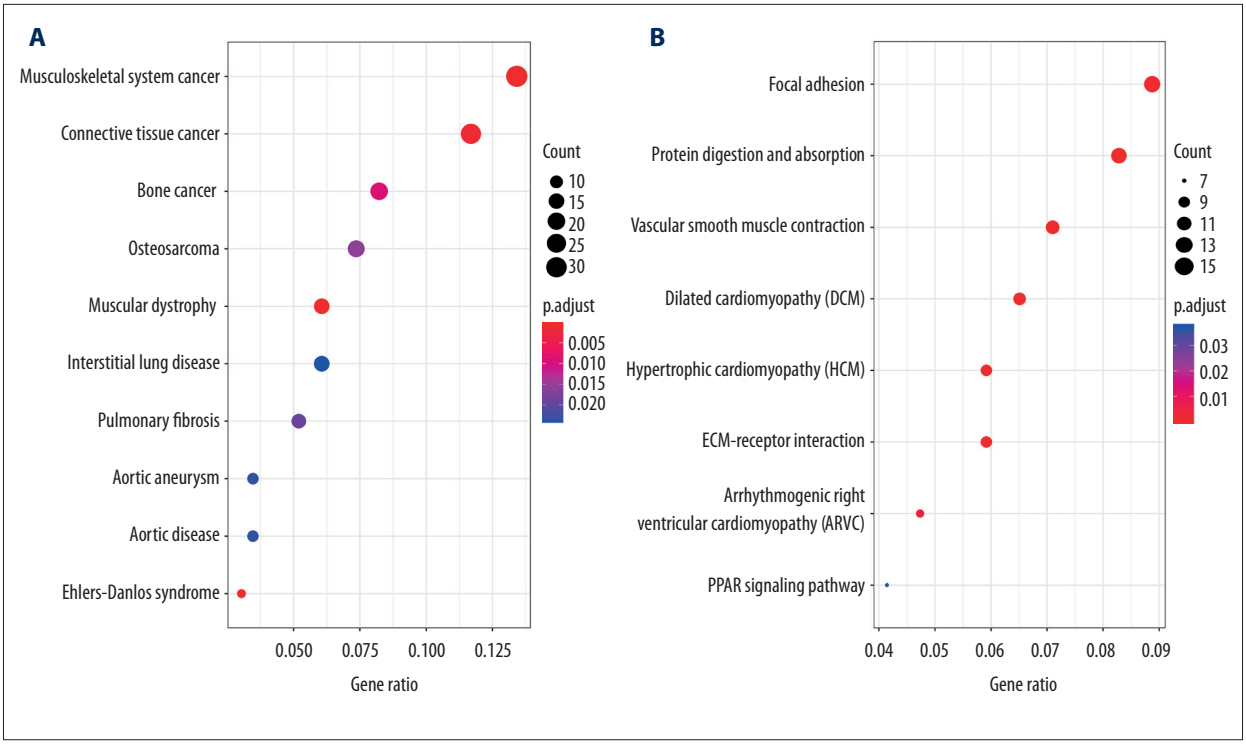

Figure 8. Disease ontology and Kyoto Encyclopedia of Genes and Genomes pathway of prognostic signature-related genes. (**A**) Disease ontology; (**B**) Kyoto Encyclopedia of Genes and Genomes pathway.

of BLCA patients. However, studies of other independent cohorts are required to validate these findings. There is no evidence that the prognostic signature can predict prognosis in patients who received adjuvant therapies after surgical resection. In the present study, the process involves mechanisms that should be validated by *in vitro* or *in vivo* experiments. Clinical information was integrated into snoRNAs expression profiles for the first time to construct a snoRNAs-based risk score by our group.

Conflict of interests

None.

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