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Prognostic value of small nuclear RNAs (snRNAs) for digestive tract pan-adenocarcinomas identified by RNA sequencing data

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ABSTRACT

Malignant tumors of the digestive tract include esophageal, gastric, and colorectal carcinomas, which all have high global mortality rates. A clinical role for small nuclear RNA (snRNA), a type of small non-coding RNA, has not yet been documented for digestive tract pan-adenocarcinomas. Therefore, the aim of the study was to identify differentially expressed snRNAs and to explore their prognostic implications in pan-adenocarcinomas from the esophagus, stomach, colon, and rectum. The pan-carcinoma RNA-sequencing data of four types of digestive tract cancers with 1, 102 cases obtained from The Cancer Genome Atlas (TCGA) project were analyzed and the differentially expressed snRNAs were evaluated using the edgeR package. The prognostic value of each of the selected snRNAs was determined by univariate and multivariate Cox regression analyses. All the digestive tract pan-adenocarcinomas showed differential expression of three snRNAs: the up-regulated RNU1-106 P and RNU6-850 P and the down-regulated RNU6-529 P. Interestingly, RNU6-101 P appeared to be a risk factor for esophageal adenocarcinoma (ESAD) and RNVU1-4 was potentially a protective factor for stomach adenocarcinoma (STAD) survival. This consistent finding of differential expression of all three snRNAs in all four types of digestive system cancers suggests potential roles for these snRNAs in the tumorigenesis of digestive system cancers. RNU6-101 P could play a pivotal role in the progression of ESAD and RNVU1-4 could perform a protective role in STAD. However, since the current findings were based on RNA-sequencing data mining, more studies are needed for verification.

1. Introduction

Malignant tumors of the digestive tract include esophageal, gastric, and colorectal carcinomas, which are common malignancies that have high global mortality rates [1–5]. The latest statistics identify digestive tract tumors as the most commonly diagnosed cancers, with colorectal carcinoma ranking third in morbidity and mortality [6–9]. Despite promising advancements in diagnosis and treatment, the prognosis of digestive tract cancers has failed to live up to the expectations of patients, which may reflect the latency and high recurrence rates of these tumors [9–14]. For this reason, research on the prognosis of the digestive tract pan-carcinomas continues to be of paramount importance. Recent research on the human genome and gene functions now indicates that RNA analysis, and particularly the study of non-coding RNAs, may be a promising avenue to follow in cancer research [15–17].

The RNA family of molecules contains the well-known protein-coding RNAs, but it also includes a number of small non-coding RNAs, such as transfer RNA (tRNA), ribosomal RNA (rRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), microRNA (miRNA), small interfering RNA (siRNA), Piwi-interacting RNA (piRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA) [15–17]. The non-coding RNAs, originally nicknamed “dark matter,” were initially considered byproducts of gene slicing and editing [18–21]. The snRNAs, which are also known as U-RNAs, are small RNA molecules about 150 nucleotides long that are detected in the splicing speckles and Cajal bodies of eukaryote cells [22–25]. In the nucleus, the snRNAs process pre-messenger RNAs (hnRNAs), regulate transcription factors (e.g., 7SK RNA) or RNA polymerase II (B2 RNA), and maintain the stability of telomeres. The snRNAs are always associated with a set of specific proteins, and the resulting complexes, termed small nuclear ribonucleoproteins (snRNPs), consist mainly of the U1, U2, U4, U5, and U6 spliceosomal RNAs. These small RNA molecules strongly influence

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Table 1
Differential expression of snRNA in esophageal adenocarcinoma.

Name	ID	Chr	logFC	logCPM	P Value	FDR
RNU6-226P	ENSG00000200648.1	chr5	−4.17932	10.1852	6.75E-18	3.98E-15
RNU6-418P	ENSG00000206762.1	chr1	−2.29433	12.79832	8.77E-12	2.58E-09
RNU2-70P	ENSG00000222650.1	chr1	−2.11031	10.97347	2.55E-08	5.01E-06
RNU6-529P	ENSG00000200253.1	chr10	−1.46665	12.04665	3.10E-07	4.56E-05
RNU1-106P	ENSG00000207110.1	chr8	2.206198	13.12236	1.21E-06	0.000143
RNVU1-8	ENSG00000201142.1	chr1	−3.69523	10.63015	3.78E-06	0.000371
RNU7-75P	ENSG00000251880.1	chr5	2.26905	11.15599	2.72E-05	0.002293
RNU1-72P	ENSG00000199846.1	chr7	2.322913	11.4096	4.11E-05	0.003026
U1	ENSG00000274428.1	chr1	3.525198	11.08494	5.86E-05	0.003832
U1	ENSG00000270722.1	chr1	3.357055	11.46246	7.18E-05	0.004229
RNU1-120P	ENSG00000199879.1	chr1	3.325177	10.6699	0.000118	0.006316
RNU6-686P	ENSG00000201709.1	chr15	−1.94542	9.972069	0.000156	0.007634
RNU6-1161P	ENSG00000251985.1	chr22	2.252146	12.30732	0.000183	0.008064
U1	ENSG00000274210.1	chr1	2.595739	12.42207	0.000192	0.008064
RNU7-143P	ENSG00000252590.1	chr7	2.535442	11.32059	0.000295	0.011571
RNU6-1340P	ENSG00000207167.1	chr16	1.69351	11.75349	0.000417	0.014771
RNU6-1048P	ENSG00000200924.1	chr2	1.358268	12.28542	0.000446	0.014771
RNU6-101P	ENSG00000222255.1	chr12	−1.29529	10.48313	0.000451	0.014771
RNU5F-1	ENSG00000199377.1	chr1	1.770491	12.37396	0.000595	0.018454
RNU6ATAC42P	ENSG00000221564.1	chr12	−1.80139	10.52062	0.000773	0.022756
RNU6-850P	ENSG00000252743.1	chr6	1.210805	13.75615	0.000857	0.023124
RNVU1-7	ENSG00000206585.1	chr1	1.864131	11.29506	0.000864	0.023124
RNU6-43P	ENSG00000207029.1	chr10	−1.44875	10.00827	0.000932	0.023867
RNU6-584P	ENSG00000222282.1	chr1	−1.2256	10.93023	0.001078	0.026449
RNU6-742P	ENSG00000202159.1	chr18	−1.0672	11.17026	0.001248	0.029392
RNU2-46P	ENSG00000252847.1	chr9	−1.34189	10.5832	0.001445	0.032552
RNU2-69P	ENSG00000251870.1	chr18	2.467976	10.33291	0.001492	0.032552
RNU6-438P	ENSG00000202431.1	chr7	3.058402	10.7989	0.001805	0.037961
RNU4-4P	ENSG00000201458.1	chr3	1.615781	11.12878	0.00212	0.04305
RNU1-129P	ENSG00000206791.1	chr1	1.770216	11.02427	0.002193	0.043056
RNU6-610P	ENSG00000206991.1	chr15	1.039524	12.04863	0.002484	0.047197

RNA biogenesis, and they direct the chemical modifications of ribosomal RNAs and other RNA genes, like those for tRNA and snRNAs [26–28], which are associated with different steps of tumorigenesis and cancer progression.

Given the common histological origination and shared molecular events among different adenocarcinomas [29], we were interested in examining the expression levels and prognostic values of snRNAs, focusing on digestive tract pan-adenocarcinomas. Here, we exploited the availability of pan-cancer RNA-sequencing data for four types of digestive tract cancers in The Cancer Genome Atlas (TCGA) project to identify snRNAs that are consistently expressed and to assess these snRNAs as prognostic indicators for digestive tract pan-adenocarcinomas from the esophagus, stomach, colon, and rectum.

2. Materials and methods

2.1. RNA-sequencing data mining from TCGA dataset for digestive tract pan-adenocarcinomas

In this study, we conducted expression profiling of mRNAs of digestive tract pan-adenocarcinomas according to sequences (Illumina Hi Seq System), survival time, and clinicopathologic parameters, all of which were available on the TCGA website. Since esophageal carcinoma includes both adenocarcinoma and squamous cell carcinoma, the data for esophageal adenocarcinoma (ESAD) were first extracted. Four types of digestive tract pan-adenocarcinomas with 1, 102 cases were examined in the current study: 80 cases of esophageal adenocarcinoma (ESAD) and 10 control cases, 375 cases of stomach adenocarcinoma (STAD) and 32 control cases, 480 cases of colon adenocarcinoma (COAD) and 41 control cases, and 167 cases of rectal adenocarcinoma (READ) and 10 control cases. All mRNA expressions were level 3 data. Since the data were provided by TCGA, no additional permission was required from the Ethics Committee. This study was also conducted in accordance with the Human Subjects Protection and Data Access Policies of TCGA.

2.2. Evaluation of the differentially expressed snRNAs in digestive tract pan-adenocarcinomas

The RNA-sequencing data contained 60,244 mRNAs. Annotation by Ensembl or NCBI yielded 1, 865 snRNAs. The edgeR package ($|\log FC| > 1$, $P < 0.05$) in R, part of the Bioconductor project, was used for variance analysis and selection of the snRNAs [30,31]. We also normalized the expression level of each snRNA (original read counts) with the edgeR package. The differentially expressed snRNAs were log2 transformed and heatmaps and volcano plots were produced using the ggplots package in R. The red and green heatmaps vividly illustrated the data distribution and facilitated cluster analysis of the data and samples. The cluster analysis data were presented as tree diagrams: the upper diagrams represented the cluster analysis of different samples from different cohorts, and the lower ones represented the cluster analysis of different genes from different samples. The most markedly changed genes were colored according to the p and FC value from the calculation of differential expression of genes.

2.3. Prognostic value of snRNAs in digestive tract pan-adenocarcinomas

The prognostic value of snRNAs was assessed with the “Survival” R package [29,32–36]. We first eliminated the differentially expressed

Table 2
Differential expression of snRNAs in stomach adenocarcinoma.

Name	ID	Chr	logFC	logCPM	PValue	FDR
RNU1-106P	ENSG00000207110.1	chr8	1.883724	13.10531	7.16E-12	3.96E-09
RNU6-387P	ENSG00000223263.1	chr1	1.304911	12.22679	1.26E-09	3.48E-07
RNU6-762P	ENSG00000252916.1	chr2	1.614896	11.80191	2.46E-09	4.54E-07
RNU6-875P	ENSG00000252297.1	chr8	2.231613	11.06662	5.12E-09	6.63E-07
RNU4-38P	ENSG00000201342.1	chr3	2.4495	11.44709	5.99E-09	6.63E-07
RNU6-610P	ENSG00000206991.1	chr15	2.059067	11.49687	8.23E-09	6.89E-07
RNU6-850P	ENSG00000252743.1	chr6	1.447493	13.41361	8.72E-09	6.89E-07
RNU7-181P	ENSG00000253043.1	chr8	1.441177	11.98499	2.75E-08	1.90E-06
RNU6-529P	ENSG00000200253.1	chr10	−1.10996	12.60846	3.39E-08	2.08E-06
RNU6-80P	ENSG00000206922.1	chr13	2.505007	10.7821	7.38E-08	3.79E-06
RNU6-925P	ENSG00000207359.1	chr8	1.263051	12.41081	7.94E-08	3.79E-06
RNU7-75P	ENSG00000251880.1	chr5	1.649392	11.30274	8.23E-08	3.79E-06
RNU6-1161P	ENSG00000251985.1	chr22	2.447986	12.38492	9.15E-08	3.89E-06
RNU1-72P	ENSG00000199846.1	chr7	2.521792	10.9897	1.26E-07	4.99E-06
RNU4-4P	ENSG00000201458.1	chr3	2.145335	10.97543	1.40E-07	5.16E-06
RNU6-669P	ENSG00000202445.1	chr9	1.905312	11.58324	2.91E-07	1.01E-05
RNU6-199P	ENSG00000199824.1	chr12	1.123262	12.29629	3.34E-07	1.09E-05
RNU1-70P	ENSG00000199488.1	chr3	−2.08503	12.12149	4.17E-07	1.28E-05
RNU6-1238P	ENSG00000253024.1	chr11	3.361094	12.27256	5.91E-07	1.72E-05
RNU7-143P	ENSG00000252590.1	chr7	3.00428	10.77043	6.94E-07	1.83E-05
RNU6-126P	ENSG00000252494.1	chr11	1.586955	11.45904	6.96E-07	1.83E-05
RNU6-583P	ENSG00000251821.1	chr4	1.955178	10.94871	1.27E-06	3.20E-05
U4	ENSG00000278374.1	chr8	1.319686	11.7869	1.86E-06	4.47E-05
RNU6-122P	ENSG00000252627.1	chrX	1.389932	11.63622	2.17E-06	4.99E-05
RNU6-403P	ENSG00000200033.1	chr1	2.585784	11.48115	2.59E-06	5.73E-05
RNU6-748P	ENSG00000207378.1	chr8	1.781361	10.88346	2.87E-06	6.11E-05
RNU6-705P	ENSG00000222533.1	chr5	2.939641	11.09201	3.05E-06	6.25E-05
RNU6-547P	ENSG00000252082.1	chr3	1.411065	11.36009	3.32E-06	6.34E-05
RNU6-1011P	ENSG00000207399.1	chr8	1.131227	11.95337	3.32E-06	6.34E-05
RNU6-438P	ENSG00000202431.1	chr7	3.464664	10.98767	3.51E-06	6.47E-05
RNU6-10P	ENSG00000206763.1	chr7	1.929241	12.12294	4.20E-06	7.49E-05
RNU6-1340P	ENSG00000207167.1	chr16	1.585555	11.62959	5.74E-06	9.92E-05
RNU6-1316P	ENSG00000206969.1	chr14	1.532821	10.97802	6.27E-06	0.000104
RNU6ATAC16P	ENSG00000221518.1	chr3	1.221488	11.50274	6.37E-06	0.000104
RNU4ATAC12P	ENSG00000252269.1	chr12	1.272241	11.84119	9.71E-06	0.000153
RNU6-1157P	ENSG00000207185.1	chr11	1.11536	11.83412	1.06E-05	0.000163
RNU4-25P	ENSG00000222501.1	chr8	1.824117	11.25874	1.22E-05	0.000183
RNU6-1048P	ENSG00000200924.1	chr2	1.317702	11.75485	1.64E-05	0.000,238
RNU6-1209P	ENSG00000200525.1	chr8	1.264834	10.89077	1.82E-05	0.000258
RNU6-930P	ENSG00000212240.1	chr6	1.052812	11.45774	2.81E-05	0.000388
RNU6-302P	ENSG00000202119.1	chr6	1.367336	10.96137	2.91E-05	0.000393
RNU5A-8P	ENSG00000200972.1	chr1	2.113913	11.47433	3.71E-05	0.000488
RNU7-18P	ENSG00000252174.1	chr3	1.588709	11.12883	3.89E-05	0.000489
RNU6-117P	ENSG00000202285.1	chr6	3.458981	10.54339	4.94E-05	0.000594
RNU6-879P	ENSG00000201198.1	chr12	1.295053	11.30362	5.29E-05	0.000623
RNU6-665P	ENSG00000207369.1	chr8	1.417416	10.86405	8.05E-05	0.000927
RNU4-80P	ENSG00000200070.1	chr15	1.27204	13.27627	8.22E-05	0.000928
RNU6-558P	ENSG00000201382.1	chr12	1.531492	11.41926	9.40E-05	0.00104
RNU1-120P	ENSG00000199879.1	chr1	1.761258	11.16943	9.92E-05	0.001076
RNU2-18P	ENSG00000223156.1	chr10	1.594932	10.62759	0.000121	0.001291
RNU6-1322P	ENSG00000201179.1	chr7	1.300234	11.11762	0.000131	0.001364
RNU6-702P	ENSG00000201852.1	chr18	1.665177	10.99194	0.000157	0.001545
RNU1-16P	ENSG00000202347.1	chr13	1.016926	11.72524	0.000157	0.001545
RNU6-141P	ENSG00000222431.1	chr6	1.840659	10.48394	0.000161	0.001545
RNU6-48P	ENSG00000206888.1	chr1	1.002003	11.10933	0.000173	0.001622
RNU6-937P	ENSG00000207053.1	chr20	1.205247	11.05949	0.000211	0.00191
RNU6-623P	ENSG00000200013.1	chr17	1.245215	10.85406	0.000233	0.002052
RNU6-377P	ENSG00000251774.1	chr3	1.008634	11.81959	0.000234	0.002052
RNU6-1337P	ENSG00000252334.1	chr19	1.243592	10.84738	0.000247	0.002132
RNU6-571P	ENSG00000206855.1	chr10	1.715053	10.5778	0.000265	0.002,233
RNU6-1128P	ENSG00000199695.1	chr22	1.179214	11.2391	0.000291	0.002343
RNU7-159P	ENSG00000238562.1	chr11	2.935975	11.15586	0.000292	0.002343
RNU7-14P	ENSG00000238468.1	chr20	2.231698	10.64061	0.000321	0.002498
RNU6-1177P	ENSG00000212360.1	chr1	1.298401	10.86012	0.000335	0.002544
RNU6-1170P	ENSG00000251783.1	chr10	1.164403	11.10561	0.000336	0.002544
RNU7-3P	ENSG00000252244.1	chr6	1.133096	11.16817	0.000407	0.002958
RNVU1-6	ENSG00000201558.1	chr1	1.365357	11.58559	0.00043	0.003049
RNU4-53P	ENSG00000222760.1	chr9	1.390808	11.11338	0.000438	0.003066
RNU6-19P	ENSG00000207449.1	chr15	1.390204	10.63028	0.000452	0.003066
RNU6-1160P	ENSG00000207276.1	chr9	1.478329	10.87144	0.000455	0.003066
RNU1-129P	ENSG00000206791.1	chr1	1.325684	10.93605	0.000485	0.003156
RNU6-249P	ENSG00000199886.1	chr12	1.479967	10.52904	0.00057	0.003574
RNU4-48P	ENSG00000202429.1	chr2	1.286828	10.70018	0.000575	0.003574
RNU1-122P	ENSG00000202408.2	chr1	1.21778	11.49457	0.000599	0.00368

(*continued on next page*)

Table 2 (*continued*)

Name	ID	Chr	logFC	logCPM	PValue	FDR
RNU6-1045P	ENSG00000252130.1	chr2	1.459595	10.45566	0.000706	0.004107
RNU1-138P	ENSG00000206820.1	chr4	1.661841	10.59594	0.000803	0.004627
RNU6-1156P	ENSG00000222297.1	chr11	1.386843	10.55338	0.000875	0.004987
RNU6-975P	ENSG00000207023.1	chr6	1.560835	10.45619	0.001037	0.005849
RNU6-1223P	ENSG00000252933.1	chr19	1.165935	11.23711	0.001058	0.005911
RNU1-92P	ENSG00000252826.1	chr1	2.172523	10.43407	0.00107	0.005919
RNU6ATAC42P	ENSG00000221564.1	chr12	1.870552	10.51511	0.00113	0.006186
RNU6-908P	ENSG00000222092.1	chr10	−1.12536	10.47774	0.001174	0.006362
RNU6-554P	ENSG00000222398.1	chr15	1.319472	10.74843	0.001295	0.006818
RNU2-52P	ENSG00000222612.1	chr20	2.006579	10.59472	0.001328	0.006926
RNVU1-4	ENSG00000277610.1	chr1	1.020934	11.84423	0.00168	0.008293
RNU6-22P	ENSG00000207083.1	chr16	1.310602	10.57419	0.001756	0.008596
RNU11-4P	ENSG00000212451.1	chr8	1.266424	10.50363	0.001774	0.008608
RNU6-570P	ENSG00000252860.1	chr1	1.35405	10.75179	0.002213	0.01055
RNU2-51P	ENSG00000222640.1	chr14	1.067413	11.09185	0.00226	0.010682
RNU6-562P	ENSG00000199601.1	chrX	1.433893	10.82486	0.003017	0.013454
RNU6-1300P	ENSG00000212348.1	chr8	1.139001	11.35884	0.003371	0.014677
RNU7-125P	ENSG00000238964.1	chr16	1.105655	10.84519	0.00342	0.014773
RNU6ATAC9P	ENSG00000252019.1	chr14	1.175785	10.78713	0.003635	0.015278
RNU6-79P	ENSG00000199381.1	chr13	1.15187	10.64463	0.003725	0.015487
RNU2-35P	ENSG00000252255.1	chr11	1.136478	10.50419	0.003966	0.016125
RNU6-1034P	ENSG00000202205.1	chr17	1.176856	10.44079	0.004	0.016147
RNU6-136P	ENSG00000207393.1	chr2	1.020523	10.65882	0.004522	0.017734
RNU4-29P	ENSG00000251752.1	chr9	1.367238	10.62909	0.004854	0.018904
RNU6-833P	ENSG00000200356.1	chr10	1.070117	10.7321	0.005255	0.019829
RNU6-1255P	ENSG00000200304.1	chr8	1.165702	10.59165	0.005549	0.020596
RNU6-1178P	ENSG00000252483.1	chr17	2.25253	10.4423	0.006607	0.023724
RNU6-924P	ENSG00000199796.1	chr19	−1.18492	10.5241	0.007274	0.025622
RNU6-640P	ENSG00000200563.1	chr2	1.083351	10.81951	0.007507	0.026273
RNU6-662P	ENSG00000206980.1	chr1	1.519789	10.55342	0.008244	0.028074
U1	ENSG00000275291.1	chr1	−1.0483	10.52821	0.008315	0.028074
RNU6-1152P	ENSG00000207306.1	chr17	1.195906	10.68738	0.008585	0.028774
RNU6-53P	ENSG00000202237.1	chr13	1.19778	10.5206	0.008789	0.029281
RNU6-218P	ENSG00000252929.1	chr7	1.046577	10.83421	0.009392	0.030198

Table 3
Differential expression of snRNA in colon adenocarcinoma.

Name	ID	Chr	logFC	logCPM	PValue	FDR
RNU6-140P	ENSG00000207296.1	chr19	−3.50631	13.88907	3.38E-62	8.58E-60
RNU6-1065P	ENSG00000207242.1	chr17	−2.67035	13.1918	2.52E-40	3.20E-38
RNU1-70P	ENSG00000199488.1	chr3	−3.93873	13.3014	3.87E-28	3.27E-26
RNU1-106P	ENSG00000207110.1	chr8	2.059494	14.81619	8.28E-18	5.26E-16
RNU6-415P	ENSG00000252061.1	chr15	−1.46148	13.73624	3.03E-17	1.54E-15
RNU6-403P	ENSG00000200033.1	chr1	4.450546	13.62117	1.89E-14	8.02E-13
RNU4-2	ENSG00000202538.1	chr12	5.684336	19.47645	1.04E-10	3.79E-09
U1	ENSG00000270722.1	chr1	4.766842	15.15735	4.87E-10	1.55E-08
RNU6-353P	ENSG00000201136.1	chr15	−1.3494	13.41591	1.03E-09	2.92E-08
RNU4-1	ENSG00000200795.1	chr12	5.385488	18.09229	1.64E-09	4.16E-08
RNU5-1	ENSG00000199347.1	chr1	5.988175	15.81551	1.91E-09	4.41E-08
RNU2-33P	ENSG00000222276.1	chr14	−1.27258	13.06232	5.91E-09	1.25E-07
RNU6-1161P	ENSG00000251985.1	chr22	2.346997	13.52618	7.22E-09	1.41E-07
RNU6-850P	ENSG00000252743.1	chr6	1.263328	14.96356	1.01E-08	1.83E-07
RNU6-722P	ENSG00000223309.1	chrX	−1.00347	14.57788	1.18E-08	2.00E-07
RNU6-137P	ENSG00000200550.1	chr2	−1.01115	13.52596	1.77E-08	2.81E-07
RNU5A-1	ENSG00000199568.1	chr15	6.677015	16.68419	3.66E-08	5.47E-07
RNU6-529P	ENSG00000200253.1	chr10	−1.25139	13.69592	4.02E-08	5.67E-07
RNU5B-1	ENSG00000200156.1	chr15	6.808827	16.54693	5.37E-07	6.67E-06
RNVU1-4	ENSG00000277610.1	chr1	1.833716	13.54195	5.48E-07	6.67E-06
RNU4ATAC	ENSG00000264229.1	chr2	2.56373	14.57948	5.52E-07	6.67E-06
U1	ENSG00000274210.1	chr1	2.389011	14.44442	5.92E-07	6.84E-06
RNU1-13P	ENSG00000238825.1	chr1	4.777644	13.68124	1.08E-06	1.19E-05
RNU7-140P	ENSG00000238364.1	chr19	−1.02767	13.13005	1.35E-06	1.42E-05
RNU1-11P	ENSG00000206702.1	chr6	5.167029	13.93648	3.20E-06	3.25E-05
RNU5A-8P	ENSG00000200972.1	chr1	3.860117	13.1571	7.26E-06	7.09E-05
RNU4-25P	ENSG00000222501.1	chr8	2.535735	12.89197	1.20E-05	0.000109
RNU5F-1	ENSG00000199377.1	chr1	2.588955	13.66777	1.51E-05	0.000132
RNU1-85P	ENSG00000200997.1	chr17	2.553184	12.74099	2.11E-05	0.00017
RNU1-88P	ENSG00000238554.1	chr6	4.952541	14.14998	2.14E-05	0.00017
RNU2-59P	ENSG00000222414.1	chr10	3.880511	13.18007	2.31E-05	0.000178
RNU6ATAC	ENSG00000221676.1	chr9	2.843662	14.40604	2.81E-05	0.00,021
RNU1-2	ENSG00000207005.1	chr1	2.945349	12.77419	3.06E-05	0.000,222

(*continued on next page*)

Table 3 (*continued*)

Name	ID	Chr	logFC	logCPM	PValue	FDR
RNVU1-7	ENSG00000206585.1	chr1	3.388633	14.37665	4.21E-05	0.000297
RNU2-6P	ENSG00000223336.1	chr13	1.63564	14.06602	4.94E-05	0.000339
RNU1-4	ENSG00000207389.1	chr1	4.762178	13.633	8.15E-05	0.000545
RNVU1-6	ENSG00000201558.1	chr1	1.952368	13.14837	0.000117	0.00074
RNU1-72P	ENSG00000199846.1	chr7	1.731238	12.88843	0.000129	0.000797
RNVU1-18	ENSG00000206737.1	chr1	3.816137	13.15123	0.00014	0.000846
RNU6-1223P	ENSG00000252933.1	chr19	1.136411	13.5462	0.000244	0.001408
U1	ENSG00000278099.1	chr1	3.657154	13.3829	0.000344	0.00186
RNU6-82P	ENSG00000200840.1	chr13	3.000899	12.81447	0.000397	0.002099
RNU6-875P	ENSG00000252297.1	chr8	1.574349	12.78463	0.000518	0.002626
RNU1-21P	ENSG00000200197.1	chr11	2.577824	13.00069	0.000527	0.002626
RNU6-438P	ENSG00000202431.1	chr7	1.424366	12.99388	0.000646	0.003157
RNU1-67P	ENSG00000207175.1	chrX	1.781606	14.18535	0.000663	0.003178
RNVU1-15	ENSG00000207205.1	chr1	1.756696	13.49334	0.000685	0.003222
RNU1-83P	ENSG00000200296.1	chr12	2.836496	12.75874	0.00079	0.003599
U1	ENSG00000277918.1	chr1	2.791928	12.96558	0.000794	0.003599
RNU1-28P	ENSG00000206588.1	chr14	3.036502	12.83749	0.000904	0.003867
RNU2-23P	ENSG00000222477.1	chr11	3.024699	12.83093	0.000913	0.003867
RNU4ATAC12P	ENSG00000252269.1	chr12	1.861192	12.73051	0.000914	0.003867
RNU5D-1	ENSG00000200169.1	chr1	2.450185	13.41006	0.001246	0.005187
RNU4-4P	ENSG00000201458.1	chr3	1.928354	12.56578	0.001635	0.006698
RNU1-89P	ENSG00000207322.1	chr4	2.68052	12.7136	0.00216	0.00871
RNU7-75P	ENSG00000251880.1	chr5	1.351251	12.92436	0.002456	0.009749
RNU6-407P	ENSG00000202150.1	chr20	1.229017	12.95866	0.003619	0.013929
RNU5-4P	ENSG00000201801.1	chr1	2.127216	12.7073	0.00375	0.01408
RNU2-61P	ENSG00000223001.1	chr6	2.844104	12.87329	0.003769	0.01408
RNU6-57P	ENSG00000223280.1	chr13	1.7118	12.56181	0.004206	0.015482
RNU6-377P	ENSG00000251774.1	chr3	1.099386	13.18812	0.004577	0.016407
RNU2-64P	ENSG00000223247.1	chr3	1.879799	12.50057	0.004586	0.016407
U7	ENSG00000272215.1	chr12	1.902669	12.50441	0.005267	0.018327
RNU1-22P	ENSG00000200204.1	chr16	1.347702	13.21105	0.006371	0.021578
RNU1-20P	ENSG00000200184.1	chr3	2.397162	12.64009	0.007202	0.024069
RNU1-19P	ENSG00000200176.1	chr10	2.143693	12.76416	0.008203	0.027058
RNU2-36P	ENSG00000222293.1	chr9	2.001323	12.536	0.008464	0.027241
RNU2-63P	ENSG00000222724.1	chr2	1.302939	13.1984	0.00858	0.027241
RNU1-52P	ENSG00000206917.1	chr17	2.264319	12.60728	0.011321	0.034898
RNU1-139P	ENSG00000212609.1	chr21	2.167334	12.58355	0.012945	0.039143
RNU6-767P	ENSG00000206859.1	chr17	1.361839	12.71138	0.013627	0.040722
RNU6-759P	ENSG00000252549.1	chr20	1.315363	12.83418	0.013799	0.040755
RNU7-124P	ENSG00000251745.1	chr9	1.83518	12.55139	0.015042	0.043417
RNU6-789P	ENSG00000252914.1	chr3	1.620109	12.51321	0.015379	0.043891
RNU1-87P	ENSG00000200597.1	chr6	1.761442	12.547	0.016245	0.045089
RNU11	ENSG00000274978.1	chr1	1.317869	13.22492	0.016332	0.045089
RNU1-46P	ENSG00000207154.1	chr18	1.926156	12.52642	0.016539	0.045172

Table 4
Differential expression of snRNA in rectal adenocarcinoma.

Name	ID	Chr	logFC	logCPM	PValue	FDR
RNU6-140P	ENSG00000207296.1	chr19	−3.59375	14.21456	5.72E-22	6.12E-20
RNU6-415P	ENSG00000252061.1	chr15	−2.04527	14.18349	2.53E-13	1.35E-11
RNU1-106P	ENSG00000207110.1	chr8	2.674928	15.26137	4.19E-07	1.49E-05
RNU6-137P	ENSG00000200550.1	chr2	−1.56361	14.01924	1.65E-06	4.10E-05
RNU6-850P	ENSG00000252743.1	chr6	2.027532	15.54662	1.91E-06	4.10E-05
RNU6-8	ENSG00000202337.1	chr14	−1.34107	15.19926	0.000223	0.003977
RNU6-481P	ENSG00000206921.1	chr1	−1.38472	13.67289	0.000321	0.004461
RNU6-1223P	ENSG00000252933.1	chr19	2.51673	14.02886	0.000334	0.004461
RNU6-1065P	ENSG00000207242.1	chr17	−1.57714	13.61015	0.000424	0.005046
RNU6-722P	ENSG00000223309.1	chrX	−1.05977	15.12894	0.001053	0.010399
RNU6-45P	ENSG00000207200.1	chr11	3.267872	13.53279	0.001069	0.010399
RNU7-49P	ENSG00000251991.1	chr11	−1.32677	13.70411	0.001609	0.014345
RNU4-47P	ENSG00000222808.1	chr17	−1.12994	14.16308	0.002232	0.015784
RNU6-353P	ENSG00000201136.1	chr15	−1.23301	13.9059	0.002259	0.015784
RNU6-377P	ENSG00000251774.1	chr3	2.576251	13.61765	0.00227	0.015784
U1	ENSG00000270722.1	chr1	2.900964	14.28562	0.002768	0.01742
U1	ENSG00000206828.1	chr1	1.293646	14.64256	0.005367	0.031905
RNU1-22P	ENSG00000200204.1	chr16	2.914798	13.7486	0.005931	0.033403
RNU6-403P	ENSG00000200033.1	chr1	2.900123	14.31148	0.006879	0.035826
RNU6-529P	ENSG00000200253.1	chr10	−1.11566	14.11292	0.007031	0.035826
RNU6-762P	ENSG00000252916.1	chr2	1.274281	14.44521	0.009026	0.043898

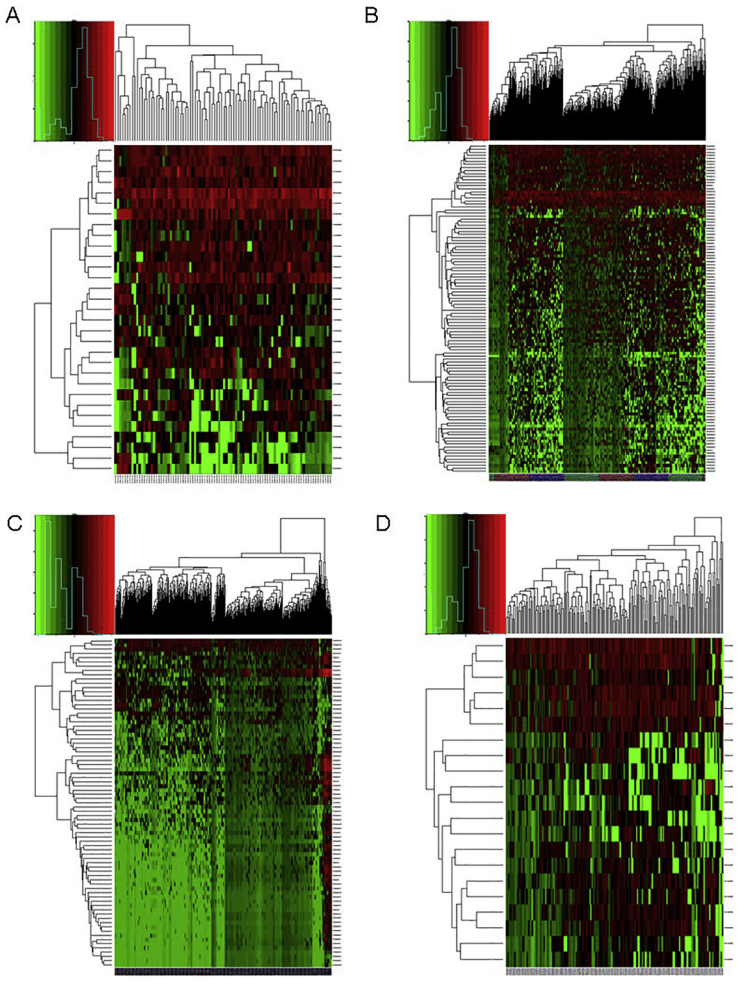

Fig. 1. Heatmaps showing differential expression of snRNA in digestive tract pan-adenocarcinomas.
Each heatmap was drawn using the R language ggplots package. The differential expression of snRNA was screened using the edgeR package (|logFC| > 1, P < 0.05). A: esophageal adenocarcinoma; B: stomach adenocarcinoma; C: colon adenocarcinoma; D: rectal adenocarcinoma.

snRNAs with expressions levels below 1 in more than 10% of the subjects. Univariate Cox regression was then performed for these differentially expressed snRNAs detected in ESAD, STAD, COAD, and READ to investigate their relationships with the survival time and survival condition. The prognostic factors (P < 0.05) that affected the patient survival were selected for further multivariate Cox analysis. The Kaplan-Meier (K–M) curve was employed to estimate the differences in the survival rate between the high-risk and low-risk cohorts.

3. Results

3.1. Differentially expressed snRNAs in digestive tract pan-adenocarcinomas

We obtained a total of 19 up-regulated and 12 down-regulated snRNAs in ESAD, 103 up-regulated and 5 down-regulated snRNAs in STAD, 67 up-regulated and 10 down-regulated snRNAs in COAD, and 10 up-regulated and 11 down-regulated snRNAs in READ (Tables 1–4, respectively). The variance analyses were also illustrated as heatmaps (Fig. 1) and volcano plots (Fig. 2).

3.2. Common differentially expressed snRNAs detected in all four digestive tract pan-adenocarcinoma types

We overlapped the differentially expressed snRNAs observed in ESAD, STAD, COAD, and READ. Two snRNAs were up-regulated consistently (RNU1-106 P and RNU6-850 P), and one was down-regulated (RNU6-529 P) in all four types of digestive tract pan-adenocarcinomas (Figs. 3 and 4). We also noticed that a snRNA, U1 appeared in the lists of differentially expressed snRNAs in four subtypes of digestive tract pan-adenocarcinomas. However, the changing trends were opposite as it was upregulated in ESAD, COAD and READ, but downregulated in STAD, which suggests that U1 may play distinct role in these digestive tract cancers.

3.3. Prognosis analysis of the differentially expressed snRNAs in digestive tract pan-adenocarcinomas

For the snRNAs in ESAD, 31 differentially expressed snRNAs were required for the prognosis investigation. We matched them with the survival time and then excluded data with missing values of more than

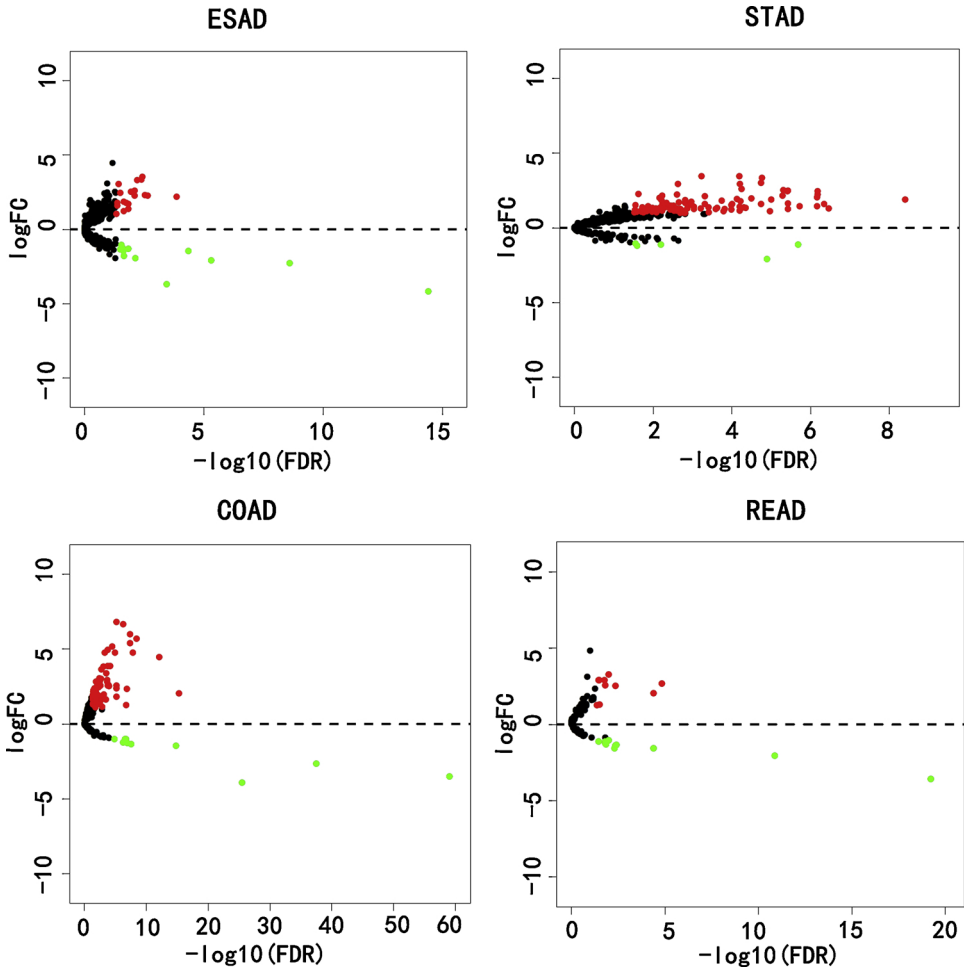

Fig. 2. Volcano plots of differential expression of snRNA in digestive tract pan-adenocarcinomas.
Volcano maps were drawn using the R language ggplots package. The difference in expression of up - regulated snRNA (logFC > 1, P < 0.05) is marked in red, and the difference in expression of down-regulated snRNA (logFC < -1, P < 0.05) is marked in green. A: esophageal adenocarcinoma B: stomach adenocarcinoma C: colon adenocarcinoma D: rectal adenocarcinoma

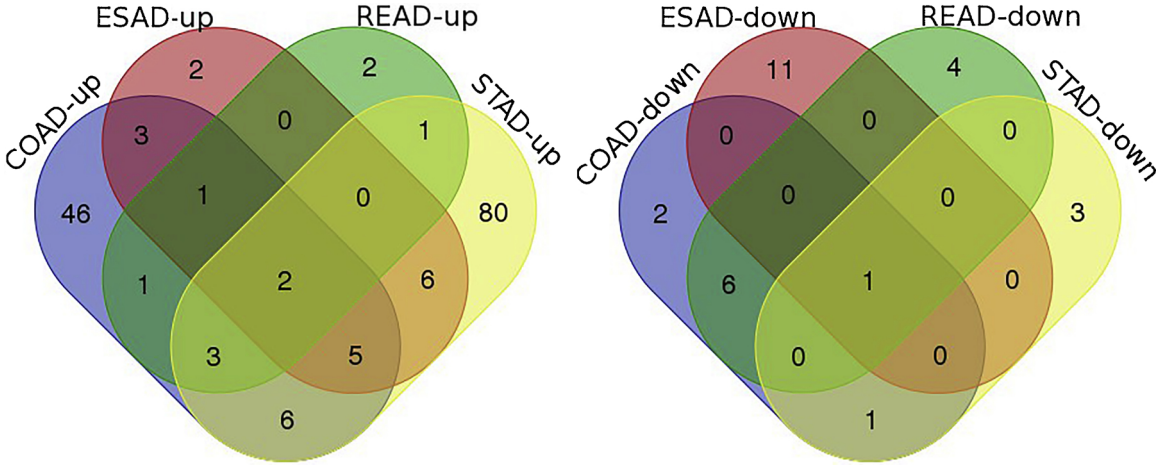

Fig. 3. Venn maps of differentially expressed snRNAs in digestive tract pan-adenocarcinomas.

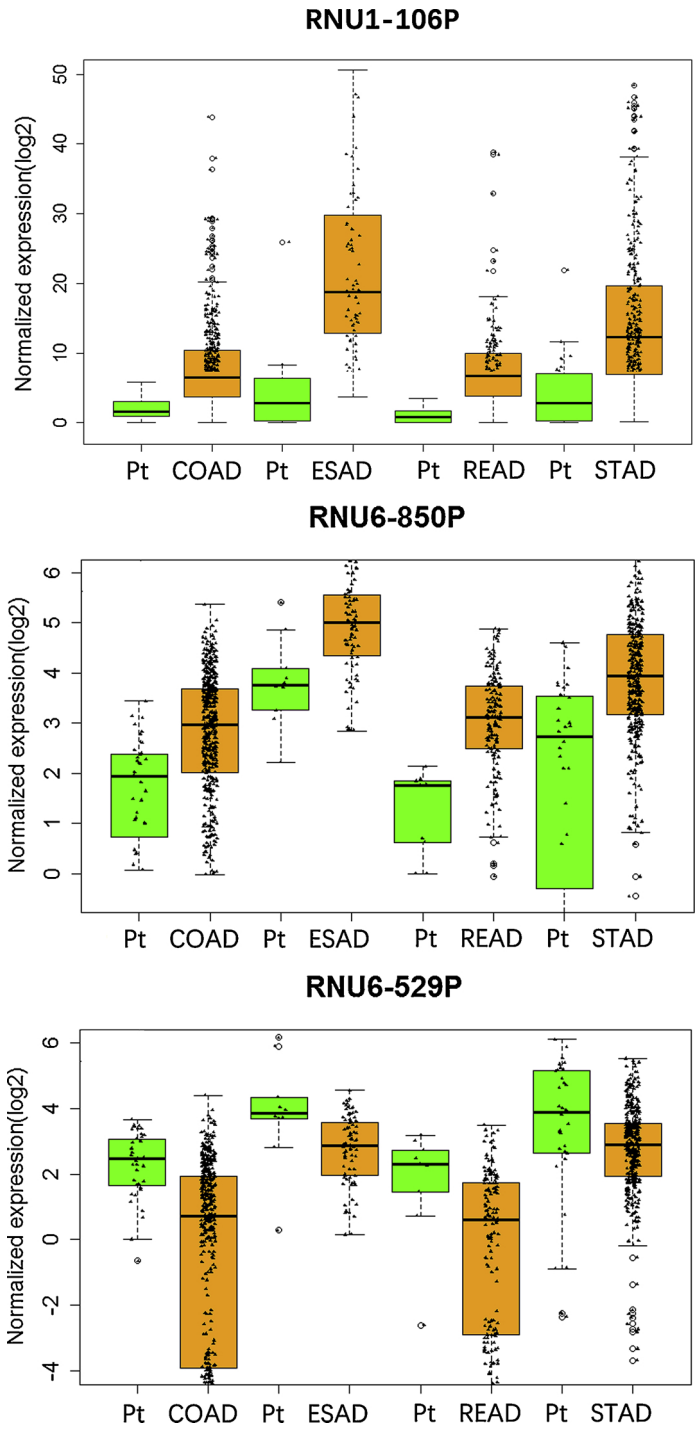

Fig. 4. Histograms of differential expression of snRNA in digestive system cancers.
In each histogram, the abscissa represents the type of cancer and the ordinate represents the log2 value of the expression. The brown yellow column represents the tumor sample, and the green represents a sample of the paracancerous control tissue. ESAD (esophageal adenocarcinoma), STAD (stomach adenocarcinoma), COAD (colon adenocarcinoma), READ (rectal adenocarcinoma)

10%, and we acquired 24 differentially expressed snRNAs. The univariate Cox regression analysis identified only one snRNA relevant to prognosis (RNU6-101 P) ($P < 0.05$) (Table 5). A subsequent investigation of the prognostic value of RNU6-101 P with the K–M curve (Fig. 5,) revealed that patients with highly expressed RNU6-101 P were more prone to poor survival than were those with lower RNU6-101 P expression, indicating that RNU6-101 P expression was a risk factor in ESAD.

Similarly, 108 differentially expressed snRNAs were obtain for STAD. Again, we matched them with the survival time and eliminated data with missing values of more than 10%, and we acquired 46 differentially expressed snRNAs. Univariate Cox regression analysis identified two prognosis-associated snRNAs, RNVU1-4 and U4 ($P < 0.05$, Table 5). The K–M curves confirmed close relationships between the expression levels of RNVU1-4, U4, and patient survival (Fig. 6–8). One snoRNA was also revealed by multivariate Cox analysis (RNVU1-4) ($P < 0.05$) (Table 5). The HR value of RNVU1-4 was -0.073, so it was considered a protective factor in STAD. Unfortunately, for the COAD

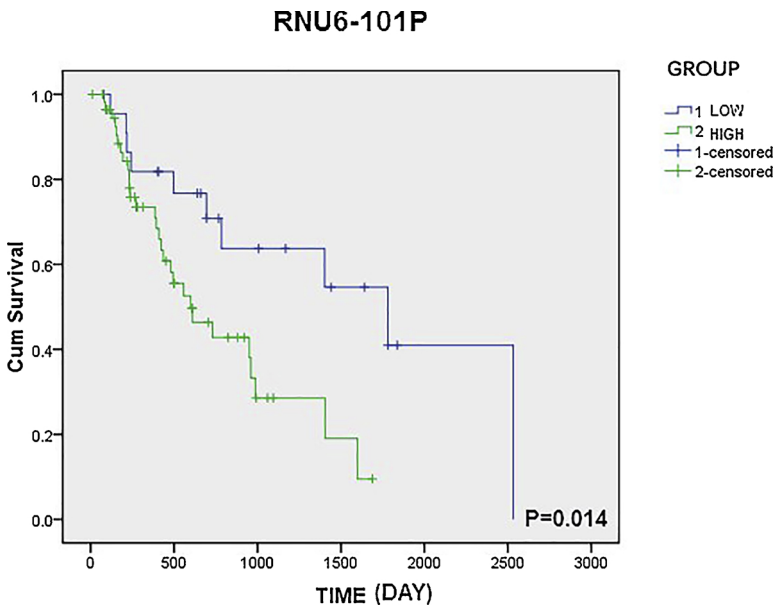

Fig. 5. The K–M survival curve for RNU6-101 P in esophageal adenocarcinoma.
K–M survival analysis was conducted using SPSS 19. The X-axis represents the survival time (days), and the Y-axis is the cumulative survival function. The blue curve represents a low-risk group, and the green a high-risk group.

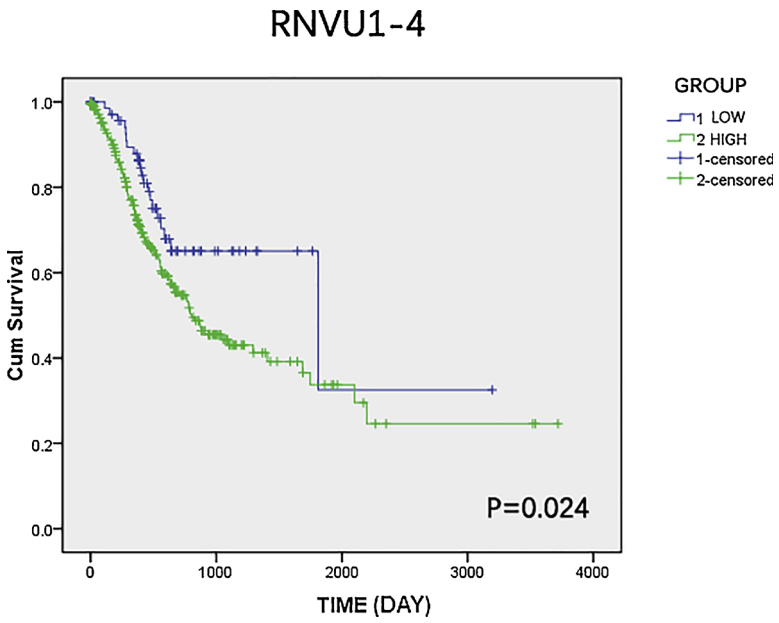

Fig. 6. The relationship between RNVU1-4 and survival in stomach adenocarcinoma (STAD).
The Kaplan-Meier (K–M) curve of RNVU1-4 expression level in STAD.

and READ groups, we failed to acquire any prognosis-related snRNAs using the univariate Cox model (data not shown).

4. Discussion

Previously, there were only sporadic reports on the clinical significance of a single snRNA in the prognosis prediction of single tumor type of the digestive system. In the current study, for the first time, 1, 102 cases of small RNA sequencing data of digestive tract pan-adenocarcinomas were re-integrated and re-analyzed to explore the prognostic value of snRNA, and plan to construct a snRNA-based prognostic assessment model. By using a variety of computational biology methods, although it was not possible to construct a prognostic model for all digestive tract pan-adenocarcinomas, we succeeded to find for

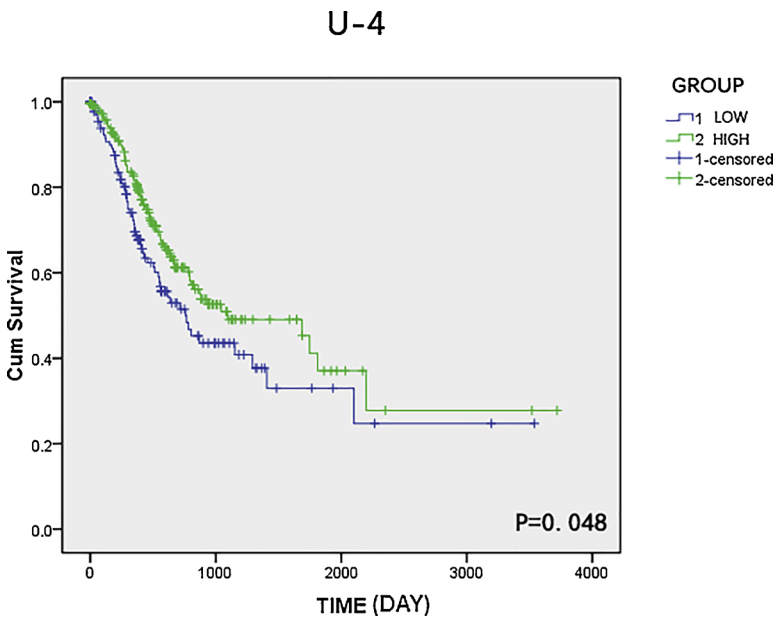

Fig. 7. The relationship between U4 and survival in stomach adenocarcinoma (STAD).
The Kaplan-Meier (K–M) curve of U4 expression level in STAD.

the first time several snRNAs with consistent differential expression level in four types of digestive tract pan-adenocarcinomas (RNU1-106 P, RNU6-850 P and RNU6-529 P). Also, we also found for the first time that RNU6-101 P in esophageal cancer and RNVU1-4 in gastric cancer may regulate the progression of the diseases and become an independent biomarker predicting patient survival.

Since 1990, the advancements in genome research and sequencing techniques have increased our knowledge of the non-coding RNAs. Several genome sequence studies have indicated that the coding RNAs account for less than 2% of the human genome. The non-coding DNA sequences, although they have no ability to encode proteins and polypeptides, exhibit their expressions in the form of non-coding RNAs [37–39]. The non-coding RNAs strongly influence biological functions and participate in the regulation of gene expression in almost all physiological or pathological processes, including embryogenesis, stem cell maintenance, cell differentiation, metabolism, signal transduction, immune response, cancers, and aging [40–42]. Non-coding RNAs are also involved in the initiation of certain severe diseases, such as tumors and cardiovascular disease. The products of the snRNAs assume housekeeping functions, but current research indicates that snRNAs and other relevant non-coding RNAs regulate various cellular functions. In particular, the expression of snRNAs greatly affects cellular homeostasis during normal growth and in response to stress [43–45]. However, the function of snRNAs and their mechanism of action in cancers are unclear.

Malignancies of the digestive system are the most common tumors encountered across the globe and have high mortality rates and poor prognosis, even after surgical resection. The National Center for Health Statistics (NCHS) has forecasted that 2018 would witness an increase of 319,160 cases in digestive system cancers (181,960 males and 137,200 females), with a death toll rising to 160,820 (94,230 males and 66,590 females). The emerging cases and the death tolls for digestive system cancers will rank first over all other malignancies [6]. In the present study, we chose four types of digestive tract pan-adenocarcinomas from the TCGA, which provided us with a large number of cases, complete gene sequencing data, and full clinical parameters and follow-up information.

Currently, the studies that have focused on snRNA are far fewer in number than those that have investigated miRNA. Roles for snRNA have been reported in certain tumors, such as breast cancer [46], prostate carcinoma [47], colorectal carcinoma [48], lung cancer [49], lymphoma [50], and ovarian cancer [51]. Considering the clinical role of snRNA in digestive tract pan-cancers, no study is available in esophageal cancer or gastric cancer but only colorectal carcinoma [43]. Koduru SV et al mined publicly published small RNA sequencing data from only eight matched colorectal samples, including benign lesions, primary cancer, and metastatic sites. They further remapped the data for various small RNA annotations including small non-coding regions of RNAs (sncRNAs) like sn/snoRNAs, mt_rRNA, misc_RNA, nonsense mediated decay and rRNAs. Finally, they identified 15 sncRNAs in the cancer vs benign lesions and 104 in the metastatic vs benign lesions, with only several being commonly expressed including RNU4-1, Y_RNA, SNORD58 A, SNORD58B and U3, among which only RNU4-1 and U3 belonged to the snRNA family [48].

Some snRNAs have also played a vital role in diagnosis. For example, in the biliary tract of patients with cholangiocarcinoma, the level of RNU2-1f was obviously higher when compared with the level in patients suffering from primary sclerosing cholangitis (AUC: 0.856, Sensitivity: 67%, Specificity: 91%) [52]. RNU2-1f was confirmed to act as a diagnostic marker of cholangiocarcinoma in the bile, and RNU2-1f was also found to function as a novel blood biomarker for pancreatic carcinoma, colorectal carcinoma, lung cancer, and melanoma [53–55]. Thus far, only a few studies have established correlations between the snRNAs and patient survival in research focused on the role of snRNA as a prognostic factor in other tumors. Research on lung cancer has revealed that the RNU2-1f functioned as a prognostic factor, as the overexpression of RNU2-1f in the serum of lung cancer patients at stage of IIIB/IV correlated with a shorter median survival time. Multiple-factor analysis further confirmed that RNU2-1f could act as an independent prognostic factor in lung cancer. Therefore, serum RNU2-1f could be employed as a biomarker for the diagnosis of lung cancer, as a prognostic indicator, and for treatment monitoring [49]. The clinical effects of snRNAs might reflect a binding deficiency of the pre-messenger RNA caused by the abnormally expressed snRNA, which has been considered to be the main cause of many cancers [56]. However, most of the molecular mechanisms involving snRNAs in cancer are unknown.

The importance of snRNAs has also been studied in some non-tumor diseases. For instance, the U1 snRNA was identified as a type of ncRNA that occurs in many diseases. For example, in Alzheimer's disease (AD)

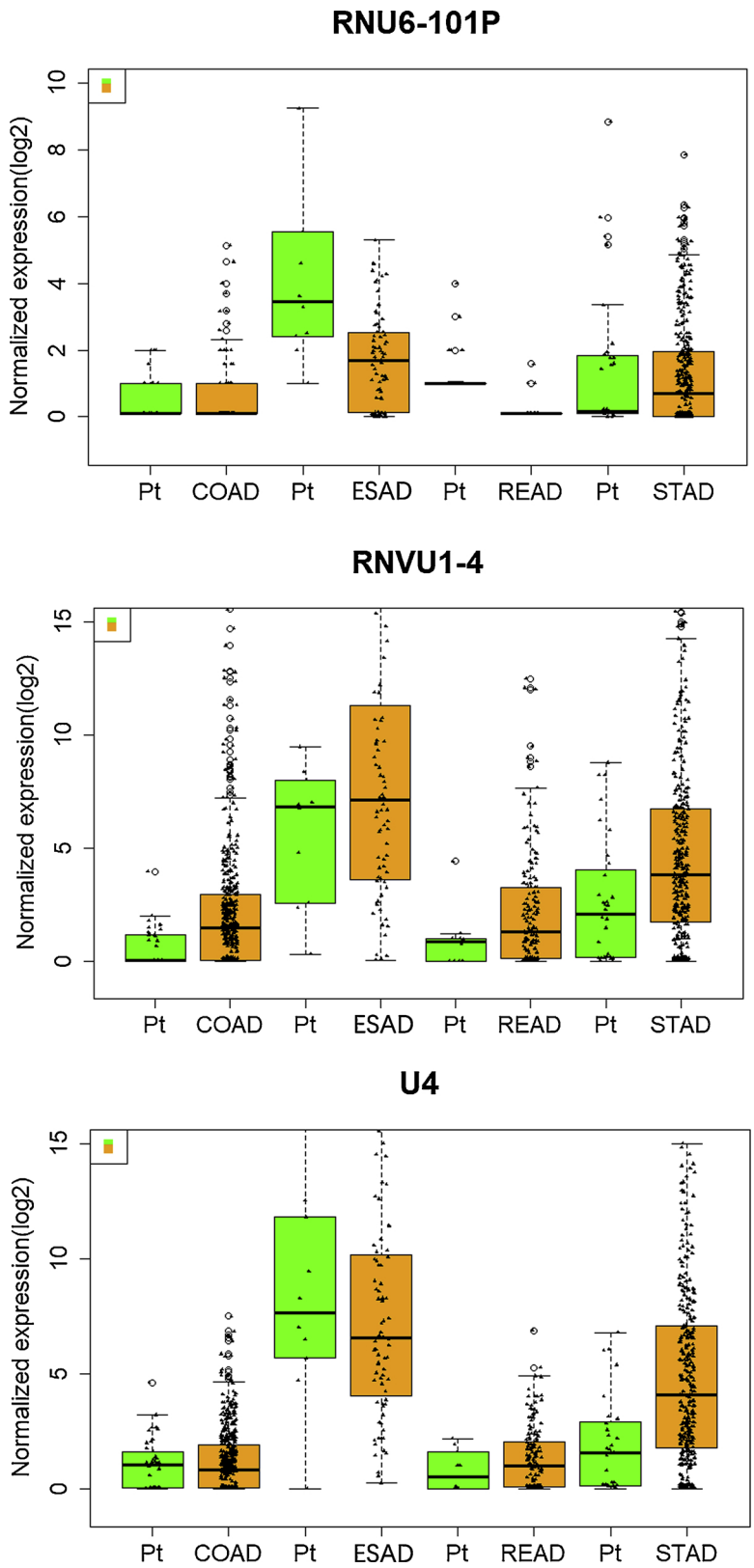

Fig. 8. Expression levels of RNU6-101 P, RNVU1-4 and U4 in four types of digestive tract pan-adenocarcinomas.
A: RNU6-101 P, B: RNVU1-4 and C: U4.

the polymerization of U1 snRNA and U1snRNPs in the cytoplasm leads to the loss of nuclear splicing, thereby altering the expressions of amyloid precursor protein and amyloid. The amyloid precursor protein has a region for the formation of amyloid, and its polymerization was regarded as a contributing factor for Alzheimer's disease [57,58].

In the present study, three differentially expressed snRNAs (RNU1-106 P, RNU6-850 P, and RNU6-529 P) were identified in all four types of digestive system tumors. RNU6-101 P appeared to be an independent

Table 5
The prognosis-related differentially expressed snRNAs in digestive tract pan-adenocarcinomas.

Disease	Cox model	Name	ID	Chr	HR	Lower	Higher	P
ESAD	Univariate	RNU6-101P	ENSG00000222255.1	Chr12	1.2408	1.045	1.473	0.014
STAD	Univariate	RNVU1-4	ENSG00000277610.1	Chr1	0.9237	0.86	0.992	0.029
STAD	Univariate	U4	ENSG00000278374.1	Chr8	0.9222	0.851	0.999	0.049
STAD	Multivariate	RNVU1-4	ENSG00000277610.1	Chr1	0.929	0.866	0.988	0.042

prognostic factor for ESAD, while RNVU1-4 was an independent prognostic factor for STAD. We had expected that several common prognostic snRNAs could be identified in the digestive tract pan-adenocarcinomas based on the consistence of the embryonic development of digestive tract organs. However, very few snRNAs could actually play a part in the progression and survival of digestive tract pan-adenocarcinomas, by far only RNU6-101 P and RNVU1-4 were achieved. Moreover, the prognostic effect of these two snRNAs are cancer type specific, which indicates that each snRNA could exert its function via distinct molecular mechanism. -Clinical roles for these snRNAs have never been reported previously for digestive tract pan-cancers. The several snRNAs reported previously [48] were not included in the current lists of differential expression snRNAs or prognostic snRNAs. This could be partially explained by the different sample sources, different patient size and different analyzing approaches. In any case, the clinical significance of the new discovered RNU1-106 P, RNU6-850 P, RNU6-529 P, RNU6-101 P, and RNVU1-4 in digestive tract pan-cancers requires further study.

In conclusion, three differentially expressed snRNAs (RNU1-106 P, RNU6-850 P, and RNU6-529 P) were found in all four digestive system cancers with 1, 102 cases investigated in the present study. More studies are needed to determine the roles of these snRNAs and their underlying mechanisms supporting tumor development. RNU6-101 P expression appears to be a risk factor for patients with ESAD, and RNVU1-4 expression could have a protective function in patients with STAD.

Conflict of interest

The authors declare no conflict of interest.

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