Small Nucleolar RNA and Small Nucleolar RNA Host Gene Signatures as Biomarkers for Pancreatic Cancer

Shuwen Han

Huzhou Central Hospital

Yangyang Xie

Ningbo No 2 Hospital

Xi Yang

Huzhou Central Hospital

Siqi Dai

Zhejiang University School of Medicine Second Affiliated Hospital

Xiaoyu Dai (✉ daixiaoyu@ucas.ac.cn)

University of the Chinese Academy of Sciences <https://orcid.org/0000-0001-7276-5190>

Research

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Abstract

Objective: The objective of this study was to identify key molecules including small nucleolar RNAs (snoRNAs) and small nucleolar RNA host genes (SNHG) involved in pancreatic cancer.

Methods: First, we screened the differentially expressed snoRNAs (DEsnoRNAs) and trend-related snoRNAs based on the cancer genome atlas (TCGA) dataset for pancreatic cancer, and then performed methylation correlation analysis, survival analysis, and extraction of snoRNA host genes for Gene ontology (GO) functional and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Next, DESNHGs and trend-related SNHGs were screened according to the TCGA dataset for pancreatic cancer, and a competing endogenous RNA (ceRNA) network was constructed for pathway and functional enrichment analysis.

Results: A total of eight DEsnoRNAs and 93 trend-related snoRNAs were extracted. Then, ten host genes of the snoRNAs were identified. Functional analysis suggested that the ten host genes were significantly enriched in several GO terms including mitotic chromosome condensation and endocytosis pathway. SNORA38B was considered to associate with survival and prognosis. The SNORD17 and SNORA11 were considered to negatively correlate with methylation. In addition, two trend-related SNHGs were extracted. Additionally, a ceRNA network was constructed with 11 miRNAs, one lncRNAs, and one mRNA. SNHG24 mainly correlated with GnRH secretion and neuroactive ligand-receptor interaction in pancreatic cancer.

Conclusion: The identified snoRNAs and SNHGs could serve as potential markers for the early detection of pancreatic cancer.

Introduction

Pancreatic cancer is one of the most common malignancies of the digestive system. It has a poor prognosis due to its short disease course, high degree of malignancy, and challenges in early detection and treatment [1]. According to statistics, only about 24% of the patients survive for one year, and 9% of the patients survive for more than five years following the diagnosis of pancreatic cancer [2]. According to GLOBOCAN (Global cancer epidemic statistics) estimates, 458,918 new cases of pancreatic cancer were diagnosed in 2018 (accounting for 2.5% of all cancers), that resulted in 438,242 deaths, accounting for 4.5% of all cancer-related deaths [3]. At present, due to the low efficiency of surgical resection and chemotherapy, and the incidence and mortality rate of pancreatic cancer continue to be on the rise [4]. Therefore, pancreatic cancer is one of the most significant cancers affecting the health of the people in China and worldwide, which has caused serious economic burden to the families of the patients [5, 6]. Therefore, new methods are needed to overcome the invasion, metastasis, and drug resistance of pancreatic cancers.

Several studies have investigated the possible molecular mechanisms of pancreatic cancer initiation and development to identify novel therapeutic targets for pancreatic cancer [7, 8]. Notably, noncoding RNA transcripts such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) have been reported to play significant roles in the molecular mechanism of cancers [9, 10]. According to the competing endogenous RNA (ceRNA) hypothesis, different RNA transcripts participate in pathological processes mainly through competing for the binding sites of shared miRNAs. Fu et al. [11] analyzed the relationship between the expression of the lncRNA, HOTTIP, and overall or disease-free survival of 90 patients with pancreatic cancer following radical resection. They found that HOTTIP modulates cancer stem cell properties in human pancreatic cancer by regulating HOXA9. Cheng et al. [12] investigated the functional effects and the possible mechanisms of the small nucleolar RNA host gene 7 (SNHG7) in pancreatic cancer, and found that SNHG7 was overexpressed in pancreatic cancer tissues, and knockdown of

SNHG7 suppressed pancreatic cancer cell proliferation, migration, and invasion through the miR-342-3p/ID4 axis. Small nucleolar noncoding RNAs (snoRNAs) are generally considered to be housekeeping molecules of ribosome function. In addition, subsets of snoRNAs were shown to be dysregulated in some cancers, including SNORA74B, SNORA21, U50, SNORD47, and SNORD62 [13, 14]. Alexey et al. [15] investigated the expression of six miRNAs isolated from formalin-fixed paraffin-embedded samples of pancreatic adenocarcinomas, and found that the snoRNA, U91, was a new internal control for accurate microRNA quantification in pancreatic cancer.

In this study, we screened differentially expressed snoRNAs (DEsnoRNAs) and trend-related snoRNAs based on the cancer genome atlas (TCGA) dataset for pancreatic cancer, and then performed methylation correlation analysis, survival analysis, and extraction of snoRNA host genes for Gene ontology (GO) functional and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Then, the DESNHGs and trend-related SNHGs were screened based on the TCGA dataset for pancreatic cancer, and a ceRNA network was constructed for pathway and functional enrichment analysis. A flow chart depicting the schematic of this study is shown in Fig. 1. This study aims to provide better understanding and promising therapeutic targets for pancreatic cancer.

Materials And Methods

snoRNA

Data preprocessing

The TCGA dataset for pancreatic cancer and the corresponding comment files were downloaded from UCSC Xena [16]. First, the data was re-annotated to extract the expression data for snoRNAs. Then the downloaded data was transformed to get the count values, and the count values were sorted to obtain the expression matrix of the data. Lastly, details about the pancreatic cancer of the sample and the stage grouping were obtained. The grouping of pancreatic cancer included tumor and normal time-series analysis of the stage.

Identification of differentially expressed snoRNAs

The differential analysis compared the tumor group with the normal. First, based on the read count data of the snoRNAs, the TMM (trimmed mean of m values) normalization provided by the R software package edge [17, 18] was used to preprocess the read counts. Then the quasi-likelihood (QL) F-test in the edgeR package was applied for screening the differentially expressed snoRNAs (DEsnoRNAs), with the screening criteria set as $p < 0.05$, $|\log FC| > 1$ (foldchange > 1.2). Based on the DEsnoRNA values obtained from the screening, a heat map was drawn to observe the clustering of samples, and a volcano plot was drawn to show the difference.

Identification of trend-related snoRNAs

Based on the classification of the tumor stage diagnosis, including stage I, stage II, stage III, and stage IV, the data were analyzed using the STEM [19] software (version 1.3.11, <http://www.cs.cmu.edu/~jernst/stem/>) for trend analysis. The data were processed with 'No normalization/add 0' and screened with $p < 0.05$.

Identification of host genes of DEsnoRNAs and trend-related snoRNAs

The host genes of DEsnoRNAs and trend-related snoRNAs in the data were extracted using the snoopy [20] tool (<http://snoopy.med.miyazaki-u.ac.jp/>).

GO functional and pathway enrichment analysis

According to the host genes of the snoRNAs, the clusterprofiler tool [21] was used to perform GO functional and KEGG pathway analysis. The GO functional analysis included biological processes (BP), cellular components (CC), and molecular functions (MF).

Survival analysis

Survival analysis was conducted on the DEsnoRNA and trend-related snoRNA data. $p < 0.05$ was considered to be an association with survival and prognosis. During the analysis, samples with survival information were screened and those with survival time longer than one month were selected.

Methylation correlation analysis

The methylation correlation analysis was conducted using the DEsnoRNAs, trend-related snoRNAs, and the data of TCGA methylation with corresponding pancreatic cancer. Then the methylation correlation results were obtained using the SNORic tool [22] (<http://bioinfo.life.hust.edu.cn/SNORic/>).

SNHG

Data preprocessing

The TCGA dataset for pancreatic cancer and the corresponding comment files were downloaded from UCSC Xena [16]. First, the data was re-annotated to extract the expression data for small nucleolar RNA host gene (SNHG), and the mRNA expression data corresponding to each dataset was extracted. The downloaded data were transformed to get the count values, and the count values were sorted to obtain the expression matrix of the data. Finally, details about the pancreatic cancer of the sample and the stage grouping were obtained. The grouping of pancreatic cancer included tumor and normal time-series analysis of stage analysis.

Identification of differentially expressed SNHGs

Differential analysis was used to compare the tumor and normal groups. First, according to the read count data, the TMM (trimmed mean of m values) normalization provided by the R software package edge [17, 18] was used to preprocess the read count. Then, the quasi-likelihood (QL) F-test in the edgeR package was applied for screening the DESNHGs, and the following screening criteria were used $p < 0.05$, $|\log FC| > 0.585$ (foldchange > 1.5).

Identification of trend-related SNHGs

Based on the classification of tumor stage diagnosis, including stage I, stage II, stage III and stage IV, the data were processed using the STEM [19] software (version 1.3.11,

<http://www.cs.cmu.edu/~jernst/stem/>) for trend analysis. First, the data were processed with 'No normalization/add 0' and screened with $p < 0.05$ parameter. Then the data were processed according to the parameter 'normalize data' and screened with $p < 0.1$.

Correlation analysis

Based on the results of the differential and trend analyses, the SNHGs related to the pancreatic cancer trend were selected and combined with the mRNA expression value of corresponding datasets. LncRNA and its corresponding

mRNA in each group of data were analyzed to obtain the lncRNA-mRNA interaction pairs. The screening threshold was set at $p < 0.05$.

Prediction of lncRNA-miRNA interaction pairs

The Miranda [23] software was used to extract the lncRNA sequence in the lncRNA-mRNA interaction pairs, and to predict the target miRNA. Then the results were screened with the screening parameters -sc 140, -en -20, and the lncRNA-miRNA interaction pairs were obtained.

Prediction of mRNA-miRNA interaction pairs

The Miranda [23] software was used to extract the mRNA sequence in the lncRNA-mRNA interaction pairs, and to predict the target miRNA. Then the results were screened with the screening parameters -sc 140, -en -20, and the mRNA-miRNA interaction pairs were obtained.

Construction of ceRNA network

The interaction pairs of lncRNA-mRNA, lncRNA-miRNA, and mRNA-miRNA were integrated to construct the ceRNA network and to obtain the ceRNA interaction pairs.

GO functional and pathway enrichment analysis in ceRNA network

The genes in the ceRNA network were used to perform KEGG pathway annotation using the clusterprofiler tool [21]. Then the pathways that may be affected by the SNHGs were obtained, and the mechanism of its effect on pancreatic cancer was analyzed.

Statistical analysis

SPSS 22.0 statistical software was used for statistical processing. Continuous variables are expressed as mean $\pm$ standard deviation (mean $\pm$ SD). The enumeration data consistent with normal distribution between two groups were compared using the t test, and the comparison among groups of enumeration data were tested using

chi-square test. Statistical difference with $p < 0.05$ was considered significant.

Results

Clinical information of pancreatic cancer data samples

As shown in Table 1, the sites of pancreatic cancer included the body of pancreas, head of pancreas, overlapping lesion of pancreas, pancreas, NOS, and tail of pancreas. There was a significant difference between stage I, II, III, and IV ($p < 0.05$). The type and age of pancreatic cancer between stage I, II, III, and IV showed no significant differences.

Table 1
Clinical information of pancreatic cancer data sample

Stage		I	II	III	IV	P value
Age		65.57 ± 12.13	64.68 ± 10.97	65.67 ± 15.28	65.80 ± 4.92	0.27
Gender	male	10	83	1	2	
	female	11	65	2	3	
Site	Body of pancreas	4	9	0	1	0.005
	Head of pancreas	12	112	3	1	
	Overlapping lesion of pancreas	0	2	0	0	
	Pancreas, NOS	1	16	0	0	
	Tail of pancreas	4	9	0	3	
Type	Adenomas and Adenocarcinomas	7	21	0	2	0.68
	Cystic, Mucinous and Serous Neoplasms	1	4	0	0	
	Ductal and Lobular Neoplasms	13	122	3	3	
	Epithelial Neoplasms, NOS	0	1	0	0	
Note: The statistical difference was considered significant if p < 0.05.						

Snorna

Identification of differentially expressed snoRNAs and trend-related snoRNAs

A total of eight DEsnoRNAs were identified using the method described above with $p < 0.05$ and $|\log FC| > 1$ (foldchange > 1.2) and consisted of two upregulated and six downregulated snoRNAs. The heatmap and volcano plot of the DEsnoRNAs are shown in Fig. 2. After the data were processed with 'No normalization/add 0' and screened with $p < 0.05$, a total of 93 trend-related snoRNAs were obtained (Fig. 3, Table 2). The top 10 trend-related snoRNAs were selected for subsequent analysis.

Table 2
Identification of trend-related snoRNAs

Weight	snoRNA	Spot IDs	0	i	ii	iii	iv
1.00	ACA59	1	0.00	8.94	9.05	10.59	10.87
1.00	SCARNA10	3	0.00	10.87	9.11	8.90	10.29
1.00	SCARNA12	4	0.00	13.41	13.09	13.51	13.09
1.00	SNORA11	5	0.00	10.75	10.54	10.80	10.45
1.00	SNORA12	6	0.00	11.50	10.84	11.08	11.12
1.00	SNORA13	7	0.00	12.63	12.76	12.59	12.78
1.00	SNORA14	8	0.00	4.79	6.20	12.26	4.82
1.00	SNORA14B	9	0.00	10.39	10.23	12.90	10.07
1.00	SNORA16	11	0.00	10.42	9.40	11.98	8.96
1.00	SNORA16B	12	0.00	8.97	10.06	7.78	13.58
1.00	SNORA19	13	0.00	3.04	4.60	6.70	9.25
1.00	SNORA2	14	0.00	12.29	12.12	12.32	12.32
1.00	SNORA20	15	0.00	3.71	4.75	6.08	3.83
1.00	SNORA22	16	0.00	9.67	9.87	7.35	10.71
1.00	SNORA23	17	0.00	8.27	8.42	12.46	7.88
1.00	SNORA26	18	0.00	9.81	9.54	9.68	9.60
1.00	SNORA3	21	0.00	5.60	5.52	9.09	7.76
1.00	SNORA31	22	0.00	8.65	8.85	8.76	9.06
1.00	SNORA33	23	0.00	12.04	11.91	12.10	11.89
1.00	SNORA34	24	0.00	11.18	10.02	12.46	10.05
1.00	SNORA38B	25	0.00	6.33	6.46	8.27	7.64
1.00	SNORA40	27	0.00	8.25	8.21	8.42	8.34
1.00	SNORA42	28	0.00	8.69	8.28	6.97	9.73
1.00	SNORA44	29	0.00	4.98	7.44	7.20	11.57
1.00	SNORA45B	30	0.00	12.18	12.33	8.86	13.55
1.00	SNORA46	31	0.00	11.23	10.70	11.73	11.93
1.00	SNORA47	32	0.00	7.73	7.03	4.70	9.57
1.00	SNORA48	33	0.00	4.65	5.01	5.30	4.48
1.00	SNORA51	34	0.00	6.67	8.42	9.26	9.25
1.00	SNORA53	35	0.00	12.56	12.85	13.51	13.32

Weight	snoRNA	Spot IDs	0	i	ii	iii	iv
1.00	SNORA54	36	0.00	7.31	8.25	8.44	7.28
1.00	SNORA55	37	0.00	11.61	12.49	8.74	14.56
1.00	SNORA58	38	0.00	9.68	8.13	6.74	8.45
1.00	SNORA59A	39	0.00	11.39	12.06	12.27	12.26
1.00	SNORA5A	40	0.00	13.11	11.86	9.11	13.09
1.00	SNORA5C	41	0.00	14.83	14.80	13.70	14.60
1.00	SNORA60	42	0.00	14.14	13.36	13.45	13.85
1.00	SNORA63	43	0.00	8.17	7.49	5.91	8.93
1.00	SNORA65	44	0.00	15.08	14.69	15.16	14.96
1.00	SNORA66	45	0.00	13.96	14.24	13.42	14.66
1.00	SNORA67	46	0.00	6.65	5.54	6.25	8.45
1.00	SNORA68	47	0.00	6.58	6.64	9.04	7.47
1.00	SNORA69	48	0.00	10.52	10.74	12.28	11.33
1.00	SNORA7	49	0.00	10.53	9.90	10.77	10.65
1.00	SNORA70F	51	0.00	5.13	7.85	12.26	12.20
1.00	SNORA71A	52	0.00	12.55	12.87	13.42	13.90
1.00	SNORA71B	53	0.00	13.20	13.28	13.53	14.19
1.00	SNORA71C	54	0.00	11.40	12.94	8.97	13.75
1.00	SNORA72	55	0.00	7.19	5.76	5.70	6.73
1.00	SNORA73B	56	0.00	15.03	15.15	15.46	15.45
1.00	SNORA74	57	0.00	6.29	5.86	5.85	5.96
1.00	SNORA75	59	0.00	9.28	9.78	9.62	9.57
1.00	SNORA77	60	0.00	7.59	9.65	10.57	9.76
1.00	SNORA81	64	0.00	10.37	10.78	10.93	10.75
1.00	SNORA9	65	0.00	8.61	9.07	10.02	9.91
1.00	SNORD100	66	0.00	14.40	14.49	14.58	14.86
1.00	SNORD101	67	0.00	12.34	12.31	12.98	13.69
1.00	SNORD104	68	0.00	16.64	16.58	16.44	16.18
1.00	SNORD11	69	0.00	6.57	6.37	3.39	10.26
1.00	SNORD116-24	70	0.00	8.69	9.22	9.05	13.32
1.00	SNORD116-4	71	0.00	8.34	9.42	8.61	11.80

Weight	snoRNA	Spot IDs	0	i	ii	iii	iv
1.00	SNORD117	72	0.00	8.14	10.08	8.64	13.22
1.00	SNORD12B	75	0.00	10.69	12.83	9.30	14.29
1.00	SNORD12C	76	0.00	6.49	7.27	8.08	9.58
1.00	SNORD14A	77	0.00	15.81	15.51	15.53	14.92
1.00	SNORD14E	78	0.00	13.59	14.07	15.11	13.54
1.00	SNORD15B	80	0.00	11.15	12.10	12.90	13.06
1.00	SNORD17	81	0.00	13.88	14.27	14.58	14.47
1.00	SNORD19	82	0.00	12.39	12.17	11.55	12.85
1.00	SNORD1B	83	0.00	5.66	6.67	7.67	6.98
1.00	SNORD46	85	0.00	10.46	10.74	10.30	11.47
1.00	SNORD51	86	0.00	6.73	8.46	7.78	11.04
1.00	SNORD53_SNORD92	87	0.00	6.16	6.56	3.85	9.48
1.00	SNORD56	88	0.00	8.90	8.99	9.60	9.77
1.00	SNORD59A	89	0.00	8.54	9.87	8.27	12.78
1.00	SNORD6	90	0.00	14.68	14.81	14.32	14.58
1.00	SNORD60	91	0.00	11.76	11.36	10.55	11.73
1.00	SNORD62B	92	0.00	13.01	12.67	13.32	13.73
1.00	SNORD63	93	0.00	10.89	11.22	11.23	11.92
1.00	SNORD67	94	0.00	4.55	6.08	7.09	4.54
1.00	SNORD69	95	0.00	12.30	12.42	12.26	13.54
1.00	SNORD72	97	0.00	6.93	9.79	12.59	10.43
1.00	SNORD83A	99	0.00	11.45	12.03	12.98	10.20
1.00	SNORD88A	101	0.00	5.11	6.10	4.15	12.26
1.00	SNORD89	102	0.00	13.28	14.16	14.52	14.09
1.00	SNORD9	103	0.00	8.71	7.72	8.30	9.55
1.00	SNORD91A	104	0.00	7.16	6.47	7.09	10.49
1.00	SNORD94	106	0.00	14.70	14.73	15.38	15.26
1.00	SNORD99	107	0.00	14.86	15.30	14.47	15.49
1.00	SNOU109	108	0.00	10.27	9.97	10.31	10.29
1.00	SNOU13	109	0.00	8.31	8.38	8.27	8.81
1.00	SNOZ196	110	0.00	11.66	12.09	8.52	12.84

Weight	snoRNA	Spot IDs	0	i	ii	iii	iv
1.00	U3	112	0.00	9.00	8.73	8.68	8.88

Identification of host genes of DEsnoRNAs and trend-related snoRNAs

In total, four host genes of the DEsnoRNAs in the data were obtained, including *NCAPD2*, *C1orf79*, *NAP1L4*, and *SNX5*. A total of six host genes of the trend-related snoRNAs in data were identified, including *NCAPD2*, *PHB2*, *MAGED2*, *CWF19L1*, *C5orf26*, and *C1orf79* (Table 3).

Table 3
Host genes of DEsnoRNAs and trend-related snoRNAs

snoRNA name	Species	snoRNA name	Box	Target RNA	Organization	Locus	name2
DEsnoRNAs							
SCARNA10	Homo_sapiens	SCARNA10 (U85)	"H/ACA,C/D"	U5 snRNA	Intronic	NCAPD2	U85
SNORA44	Homo_sapiens	SNORA44 (ACA44)	H/ACA	18S rRNA	Intronic	C1orf79	ACA44
SNORA54	Homo_sapiens	SNORA54 (ACA54)	H/ACA	28S rRNA	Intronic	NAP1L4	ACA54
SNORD17	Homo_sapiens	SNORD17 (HBI-43)	C/D	28S rRNA	Intronic	SNX5	HBI-43
trend-related snoRNAs							
SCARNA10	Homo sapiens	SCARNA10 (U85)	"H/ACA,C/D"	U5 snRNA	Intronic	NCAPD2	U85
SCARNA12	Homo sapiens	SCARNA12 (U89)	H/ACA	U5 snRNA	Intronic	PHB2	U89
SNORA11	Homo sapiens	SNORA11 (U107)	H/ACA	Unknown	Intronic	MAGED2	U107
SNORA12	Homo sapiens	SNORA12 (U108)	H/ACA	Unknown	Intronic	CWF19L1	U108
SNORA13	Homo sapiens	SNORA13 (ACA13)	H/ACA	18S rRNA	intronic	C5orf26	ACA13
SNORA16	Homo sapiens	SNORA16 (ACA16)	H/ACA	28S rRNA	Intronic	C1orf79	ACA16

Functional analysis of host genes of DEsnoRNAs and trend-related snoRNAs

The GO enrichment analysis of the host genes of DEsnoRNAs revealed that the significantly enriched GO-BP terms included mitotic chromosome condensation, chromosome condensation, and mitotic sister chromatid segregation. The condensin complex, DNA packaging complex, and condensed chromosome were three markedly enriched GO-CC terms. Furthermore, we also found that these genes played essential roles in histone binding, nucleosome binding,

and actin filament binding according to the GO-MF analysis. The KEGG pathway analysis suggested that these genes were responsible for endocytosis pathway. The GO enrichment analysis of the host genes of trend-related snoRNAs revealed that the significantly enriched GO-BP terms included mitotic chromosome condensation, chromosome condensation, and mitotic sister chromatid segregation. The condensin complex, DNA packaging complex, and condensed chromosome were three markedly enriched GO-CC terms. Furthermore, we also found that these genes played essential roles in histone binding, nucleosome binding, and estrogen receptor binding according to the GO-MF analysis. The KEGG pathway analysis revealed that these genes were responsible for the endocytosis pathway (Fig. 4 and Supplementary file 1).

Survival analysis of DEsnoRNAs and trend-related snoRNAs

snoRNA survival analysis was performed based on the DEsnoRNAs and trend-related snoRNAs. As shown in the Fig. 5B, SNORA38B, a DEsnoRNA with $p = 0.0092$, was found to be associated with survival and prognosis. The survival analysis of the top 10 trend-related snoRNA are shown in Fig. 5.

Methylation correlation analysis of DEsnoRNAs and trend-related snoRNAs

Methylation correlation analysis was performed based on the DEsnoRNAs and trend-related snoRNAs. As illustrated in Fig. 6 and Table 4, SNORD17, a DEsnoRNA with the $p = 0.0412$ and correlation coefficient = -0.2077 , was found to be negatively correlated with methylation. SNORA11, a trend-related snoRNA, was also found to be negatively correlated with methylation ($p = 0.0336$ and correlation coefficient = -0.2159).

Table 4
Methylation correlation analysis of DEsnoRNAs and trend-related snoRNAs

snoRNA	Chip ID	Coefficient	P-value
ACA54; ENSG00000207008; SNORA54	cg26350143	0.0831	0.4187
ACA54; ENSG00000207008; SNORA54	cg03241897	-0.0234	0.8201
HBI-43; ENSG00000212232; SNORD17	cg07577824	-0.082	0.4245
HBI-43; ENSG00000212232; SNORD17	cg17507897	-0.0823	0.4228
HBI-43; ENSG00000212232; SNORD17	cg02912291	-0.1451	0.1561
HBI-43; ENSG00000212232; SNORD17	cg22145445	-0.2077	0.0412
U107; ENSG00000221716; SNORA11	cg23090195	-0.1127	0.2718
U107; ENSG00000221716; SNORA11	cg18528798	-0.1455	0.1549
U107; ENSG00000221716; SNORA11	cg14338160	-0.1818	0.0747
U107; ENSG00000221716; SNORA11	cg22072691	-0.2159	0.0336

SNHG

Identification of differentially expressed SNHGs and trend-related SNHGs

Based on $p < 0.05$, $|\log FC| > 0.585$ (foldchange > 1.5), no DESNHG was identified. After the data were processed with 'No normalization/add 0' and screened with $p < 0.05$, there were no significant trend-related SNHGs. Then the data were processed based on the parameter 'normalize data' and screened with $p < 0.1$, and two SNHGs correlated with trends, including SNHG23 and SNHG24 (Fig. 7, Table 5). The expression values of these two SNHGs were selected for subsequent analysis.

Table 5
Identification of trend-related SNHGs

Weight	stage	Spot IDs	0	stage i	stage ii	stage iii	stage iv
No normalize/add0							
1.00	SNHG16	1	0.00	15.05	15.33	15.70	15.73
1.00	SNHG11	2	0.00	14.47	14.46	14.36	14.28
1.00	SNHG17	3	0.00	15.40	15.54	15.48	15.90
1.00	SNHG12	4	0.00	14.56	15.28	14.77	15.27
1.00	SNHG5	5	0.00	17.18	17.58	18.23	17.92
1.00	SNHG14	6	0.00	16.03	15.65	15.71	15.90
1.00	SNHG23	7	0.00	8.53	7.98	9.65	8.44
1.00	SNHG15	8	0.00	15.09	15.28	15.32	15.42
1.00	SNHG7	9	0.00	16.42	16.12	16.34	16.14
1.00	SNHG20	10	0.00	13.75	13.57	13.31	13.71
1.00	SNHG3	11	0.00	15.20	15.43	15.09	15.33
1.00	SNHG6	12	0.00	16.95	16.90	17.16	16.68
1.00	SNHG10	13	0.00	13.23	13.03	12.81	13.02
1.00	SNHG18	14	0.00	13.63	14.36	14.57	14.41
1.00	SNHG21	15	0.00	11.26	11.10	11.25	10.96
1.00	SNHG9	16	0.00	13.27	12.74	12.81	12.65
1.00	SNHG1	17	0.00	15.89	16.25	15.99	16.34
1.00	SNHG19	18	0.00	14.55	13.90	13.83	13.89
1.00	SNHG22	19	0.00	10.71	10.84	10.58	10.44
1.00	SNHG8	20	0.00	16.90	16.48	16.80	16.57
1.00	SNHG24	21	0.00	7.59	7.22	7.92	6.92
1.00	SNHG4	22	0.00	10.70	11.84	10.96	11.93
Normalize							
1.00	SNHG23	7			0.00	-0.55	1.13 -0.09
1.00	SNHG24	21			0.00	-0.37	0.33 -0.67

Identification of lncRNA-mRNA interaction pairs

Based on the SNHG24 related to the pancreatic cancer trend, the data of the lncRNA and the corresponding mRNAs in each group were analyzed with the screening threshold $p < 0.05$. The result revealed two lncRNA-mRNA interaction pairs, including SNHG24-FAM25A and SNHG24-KISS1.

Prediction of lncRNA-miRNA and mRNA-miRNA interaction pairs

With the screening parameters of -sc 140 and -en -20, a total of 127 lncRNA-miRNA interaction pairs were obtained. The lncRNA-miRNA interaction pairs are shown in Table 6. With the screening parameters of -sc 140 and -en -20, a total 99 mRNA-miRNA interaction pairs were identified. The mRNA-miRNA interaction pairs are shown in Table 7.

Table 6
Prediction of lncRNA-miRNA interaction pairs

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-17-5p	SNHG24	169	-24.75	169	-24.75	19	23	1634	879
hsa-miR-19b-1-5p	SNHG24	149	-26.29	149	-26.29	25	23	1634	775
hsa-miR-20a-5p	SNHG24	165	-21.46	165	-21.46	28	23	1634	879
hsa-miR-26a-1-3p	SNHG24	159	-20.94	159	-20.94	42	22	1634	447
hsa-miR-93-5p	SNHG24	169	-25.95	169	-25.95	62	23	1634	879
hsa-miR-103a-3p	SNHG24	140	-20.88	140	-20.88	80	23	1634	662
hsa-miR-106a-5p	SNHG24	169	-20.51	169	-20.51	84	23	1634	879
hsa-miR-107	SNHG24	140	-20.88	140	-20.88	86	23	1634	662
hsa-miR-23b-5p	SNHG24	143	-21.29	143	-21.29	177	22	1634	1441
hsa-miR-132-3p	SNHG24	152	-23.84	152	-23.84	194	22	1634	4
hsa-miR-140-3p	SNHG24	153	-22.18	153	-22.18	205	21	1634	246
hsa-miR-152-3p	SNHG24	145	-20.88	145	-20.88	217	21	1634	129
hsa-miR-302a-3p	SNHG24	147	-20.34	147	-20.34	278	23	1634	541
hsa-miR-99b-3p	SNHG24	150	-22.74	150	-22.74	290	22	1634	490
hsa-miR-302b-3p	SNHG24	151	-20.92	151	-20.92	309	23	1634	541
hsa-miR-378a-5p	SNHG24	140	-20.4	140	-20.4	336	22	1634	264
hsa-miR-380-5p	SNHG24	149	-21.95	149	-21.95	340	22	1634	1140
hsa-miR-135b-3p	SNHG24	140	-25.32	140	-25.32	364	22	1634	81
hsa-miR-20b-5p	SNHG24	165	-24.08	165	-24.08	394	23	1634	879
hsa-miR-484	SNHG24	153	-20.39	153	-20.39	420	22	1634	943
hsa-miR-485-5p	SNHG24	146	-21.86	146	-21.86	421	22	1634	324

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-511-5p	SNHG24	141	-27.13	141	-27.13	435	21	1634	785
hsa-miR-497-3p	SNHG24	150	-20.13	150	-20.13	454	22	1634	1081
hsa-miR-519b-3p	SNHG24	142	-20.1	142	-20.1	476	22	1634	128
hsa-miR-518a-5p	SNHG24	145	-20.32	145	-20.32	508	20	1634	798
hsa-miR-527	SNHG24	145	-20.32	145	-20.32	518	20	1634	798
hsa-miR-501-3p	SNHG24	155	-20.7	155	-20.7	527	22	1634	1030
hsa-miR-450a-2-3p	SNHG24	143	-22.98	143	-22.98	530	22	1634	1317
hsa-miR-585-5p	SNHG24	164	-28.23	164	-28.23	607	22	1634	262
hsa-miR-642a-5p	SNHG24	163	-21.41	163	-21.41	688	22	1634	1166
hsa-miR-657	SNHG24	147	-22.56	147	-22.56	721	23	1634	770
hsa-miR-758-3p	SNHG24	141	-20.22	141	-20.22	731	22	1634	1415
hsa-miR-1301-3p	SNHG24	145	-20.86	145	-20.86	753	24	1634	84
hsa-miR-762	SNHG24	147	-30.34	147	-30.34	767	22	1634	326
hsa-miR-759	SNHG24	142	-21.54	142	-21.54	776	22	1634	422
hsa-miR-942-5p	SNHG24	149	-24.42	149	-24.42	846	22	1634	1210
hsa-miR-1204	SNHG24	146	-22.06	146	-22.06	884	21	1634	485
hsa-miR-1304-5p	SNHG24	149	-25.86	149	-25.86	911	22	1634	39
hsa-miR-1249-5p	SNHG24	140	-27.44	140	-27.44	923	24	1634	557
hsa-miR-1266-5p	SNHG24	142	-23.1	142	-23.1	944	23	1634	1445
hsa-miR-1910-3p	SNHG24	165	-25.79	165	-25.79	1004	20	1634	674
hsa-miR-2278	SNHG24	142	-21.48	142	-21.48	1033	22	1634	1277
hsa-miR-2681-3p	SNHG24	147	-23.68	147	-23.68	1035	22	1634	68
hsa-miR-3125	SNHG24	156	-20.05	156	-20.05	1057	20	1634	1473

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-3127-5p	SNHG24	291	-54.17	147	-32.51	1060	23	1634	148 491
hsa-miR-378b	SNHG24	148	-22.29	148	-22.29	1070	19	1634	911
hsa-miR-548u	SNHG24	145	-22.16	145	-22.16	1087	23	1634	471
hsa-miR-3150a-5p	SNHG24	145	-25.5	145	-25.5	1098	22	1634	820
hsa-miR-3151-5p	SNHG24	283	-43.69	143	-22.62	1100	21	1634	917 1005
hsa-miR-3178	SNHG24	140	-27.17	140	-27.17	1141	17	1634	1005
hsa-miR-3180-5p	SNHG24	151	-22.38	151	-22.38	1143	25	1634	1435
hsa-miR-3187-3p	SNHG24	465	-71.69	163	-25.74	1157	20	1634	483 320 236
hsa-miR-3191-3p	SNHG24	144	-28.29	144	-28.29	1165	23	1634	1147
hsa-miR-3194-3p	SNHG24	155	-21.7	155	-21.7	1170	22	1634	775
hsa-miR-4298	SNHG24	141	-20.93	141	-20.93	1192	22	1634	1147
hsa-miR-4304	SNHG24	146	-24.11	146	-24.11	1194	17	1634	666
hsa-miR-3614-5p	SNHG24	141	-22.32	141	-22.32	1279	23	1634	910
hsa-miR-3617-3p	SNHG24	164	-21.7	164	-21.7	1285	23	1634	798
hsa-miR-3622b-3p	SNHG24	152	-23.87	152	-23.87	1296	21	1634	459
hsa-miR-3663-5p	SNHG24	292	-42.98	150	-22.86	1311	21	1634	279 1040
hsa-miR-3667-3p	SNHG24	141	-21.02	141	-21.02	1318	22	1634	1024
hsa-miR-3678-3p	SNHG24	147	-21.26	147	-21.26	1329	22	1634	1489
hsa-miR-3916	SNHG24	140	-22.49	140	-22.49	1367	26	1634	670
hsa-miR-3150b-5p	SNHG24	145	-27.52	145	-27.52	1371	22	1634	820
hsa-miR-3945	SNHG24	143	-21.37	143	-21.37	1405	23	1634	1142
hsa-miR-1268b	SNHG24	141	-28.91	141	-28.91	1415	20	1634	1000
hsa-miR-4441	SNHG24	152	-20.26	152	-20.26	1449	17	1634	658

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-4446-3p	SNHG24	141	-21.87	141	-21.87	1456	22	1634	330
hsa-miR-4462	SNHG24	145	-23.63	145	-23.63	1475	23	1634	666
hsa-miR-4470	SNHG24	297	-43.74	151	-22.13	1486	21	1634	54 1084
hsa-miR-4496	SNHG24	162	-27.52	162	-27.52	1523	22	1634	1468
hsa-miR-4501	SNHG24	296	-41.82	151	-21.78	1528	21	1634	742 350
hsa-miR-4513	SNHG24	142	-23.92	142	-23.92	1541	22	1634	1463
hsa-miR-4520-5p	SNHG24	161	-20.78	161	-20.78	1548	20	1634	693
hsa-miR-4647	SNHG24	144	-21.07	144	-21.07	1608	23	1634	929
hsa-miR-4671-3p	SNHG24	142	-22.58	142	-22.58	1652	21	1634	128
hsa-miR-1343-3p	SNHG24	147	-22.41	147	-22.41	1676	22	1634	41
hsa-miR-4688	SNHG24	143	-22.64	143	-22.64	1677	22	1634	503
hsa-miR-4691-5p	SNHG24	145	-20.5	145	-20.5	1681	23	1634	1
hsa-miR-4695-5p	SNHG24	140	-25.09	140	-25.09	1688	22	1634	677
hsa-miR-4717-3p	SNHG24	149	-24.43	149	-24.43	1730	21	1634	94
hsa-miR-4732-5p	SNHG24	140	-21.3	140	-21.3	1756	23	1634	746
hsa-miR-4734	SNHG24	143	-25.03	143	-25.03	1760	22	1634	772
hsa-miR-4739	SNHG24	146	-20.02	146	-20.02	1769	25	1634	648
hsa-miR-4756-5p	SNHG24	146	-20.67	146	-20.67	1802	23	1634	651
hsa-miR-2467-5p	SNHG24	140	-23.08	140	-23.08	1855	23	1634	36
hsa-miR-2467-3p	SNHG24	145	-24.03	145	-24.03	1856	22	1634	502
hsa-miR-4793-5p	SNHG24	159	-25.79	159	-25.79	1867	24	1634	37
hsa-miR-4802-3p	SNHG24	168	-26.67	168	-26.67	1884	23	1634	904
hsa-miR-5088-5p	SNHG24	283	-57.68	143	-29.23	1918	24	1634	239 488

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-5189-3p	SNHG24	141	-21.09	141	-21.09	1932	21	1634	825
hsa-miR-5683	SNHG24	154	-20.92	154	-20.92	1996	24	1634	255
hsa-miR-5691	SNHG24	155	-24.01	155	-24.01	2006	22	1634	775
hsa-miR-6079	SNHG24	162	-20.2	162	-20.2	2042	24	1634	222
hsa-miR-6503-5p	SNHG24	470	-66	173	-25.22	2078	23	1634	1036 696 862
hsa-miR-6506-5p	SNHG24	145	-20.49	145	-20.49	2084	23	1634	1148
hsa-miR-6730-5p	SNHG24	143	-22.1	143	-22.1	2130	23	1634	573
hsa-miR-6739-3p	SNHG24	141	-23.61	141	-23.61	2149	21	1634	780
hsa-miR-6743-5p	SNHG24	147	-26.79	147	-26.79	2156	22	1634	673
hsa-miR-6753-5p	SNHG24	145	-20.88	145	-20.88	2175	22	1634	654
hsa-miR-6754-5p	SNHG24	145	-21.62	145	-21.62	2177	22	1634	652
hsa-miR-6798-5p	SNHG24	143	-23.26	143	-23.26	2265	23	1634	422
hsa-miR-6805-3p	SNHG24	154	-25.43	154	-25.43	2280	23	1634	774
hsa-miR-6812-5p	SNHG24	143	-30.53	143	-30.53	2293	25	1634	996
hsa-miR-6815-5p	SNHG24	144	-24.08	144	-24.08	2299	23	1634	321
hsa-miR-6818-3p	SNHG24	152	-20.67	152	-20.67	2306	22	1634	787
hsa-miR-6819-5p	SNHG24	140	-23.87	140	-23.87	2307	22	1634	1002
hsa-miR-6820-3p	SNHG24	147	-21.1	147	-21.1	2310	22	1634	1415
hsa-miR-6847-5p	SNHG24	321	-45.97	165	-24.03	2363	23	1634	28 1471
hsa-miR-6848-3p	SNHG24	140	-20.26	140	-20.26	2366	19	1634	953
hsa-miR-6852-5p	SNHG24	149	-29.16	149	-29.16	2373	21	1634	39

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-6861-3p	SNHG24	140	-20.85	140	-20.85	2393	18	1634	1453
hsa-miR-6865-5p	SNHG24	142	-20.95	142	-20.95	2399	23	1634	322
hsa-miR-6866-5p	SNHG24	145	-21.27	145	-21.27	2401	23	1634	503
hsa-miR-6873-5p	SNHG24	163	-26.55	163	-26.55	2415	22	1634	424
hsa-miR-6874-5p	SNHG24	143	-25.2	143	-25.2	2417	23	1634	74
hsa-miR-6893-5p	SNHG24	145	-21.66	145	-21.66	2455	21	1634	673
hsa-miR-7107-3p	SNHG24	153	-22.93	153	-22.93	2464	27	1634	860
hsa-miR-7157-3p	SNHG24	305	-42.06	155	-21.29	2493	22	1634	519 608
hsa-miR-1273 h-3p	SNHG24	150	-22.22	150	-22.22	2515	22	1634	837
hsa-miR-7974	SNHG24	143	-22.14	143	-22.14	2533	24	1634	351
hsa-miR-8087	SNHG24	146	-27.19	146	-27.19	2573	23	1634	473
hsa-miR-10394-5p	SNHG24	152	-22.84	152	-22.84	2596	24	1634	770
hsa-miR-10394-3p	SNHG24	141	-21.6	141	-21.6	2597	23	1634	1001
hsa-miR-12115	SNHG24	145	-20.03	145	-20.03	2636	24	1634	916
hsa-miR-12117	SNHG24	170	-21.95	170	-21.95	2638	20	1634	921
hsa-miR-12122	SNHG24	150	-23.83	150	-23.83	2643	25	1634	31

Table 7
Prediction of mRNA-miRNA interaction pairs

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-149-3p	KISS1	151	-29.06	151	-29.06	480	21	143	35
hsa-miR-328-5p	KISS1	299	-66.55	159	-37.7	704	23	143	55 34
hsa-miR-135b-3p	KISS1	144	-24.63	144	-24.63	728	22	143	48
hsa-miR-486-3p	KISS1	151	-23.4	151	-23.4	848	21	143	112
hsa-miR-92b-5p	FAM25A	143	-28.11	143	-28.11	1133	22	56	5
hsa-miR-663a	KISS1	159	-32.27	159	-32.27	1412	22	143	65
hsa-miR-762	KISS1	147	-38.22	147	-38.22	1534	22	143	64
hsa-miR-675-5p	KISS1	151	-22.73	151	-22.73	1558	23	143	113
hsa-miR-744-5p	KISS1	439	-81.89	154	-28.03	1618	22	143	73 113 53
hsa-miR-873-3p	FAM25A	151	-23.09	151	-23.09	1637	22	56	13
hsa-miR-1228-5p	KISS1	149	-33.35	149	-33.35	1732	21	143	72
hsa-miR-1237-5p	KISS1	468	-89.22	171	-35.03	1752	21	143	60 67 37
hsa-miR-1305	KISS1	143	-20.38	143	-20.38	1826	22	143	15
hsa-miR-1275	FAM25A	141	-25.74	141	-25.74	1905	17	56	14
hsa-miR-1908-5p	KISS1	314	-52.63	159	-26.58	1998	21	143	70 57
hsa-miR-2110	FAM25A	154	-27.34	154	-27.34	2039	22	56	10
hsa-miR-2115-5p	KISS1	143	-23.35	143	-23.35	2046	22	143	23
hsa-miR-2682-5p	KISS1	154	-26.11	154	-26.11	2072	23	143	110
hsa-miR-3127-5p	KISS1	152	-26.39	152	-26.39	2120	23	143	81
hsa-miR-3141	KISS1	141	-30.22	141	-30.22	2168	19	143	68
hsa-miR-3151-5p	KISS1	289	-49.35	146	-27.73	2200	21	143	113 48
hsa-miR-3154	FAM25A	142	-23.45	142	-23.45	2213	22	56	6

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-3180-3p	KISS1	288	-53.98	148	-29.34	2288	22	143	65 109
hsa-miR-3191-3p	KISS1	160	-27.23	160	-27.23	2330	23	143	54
hsa-miR-3196	KISS1	292	-46.19	152	-25.88	2344	18	143	71 62
hsa-miR-4254	KISS1	140	-23.26	140	-23.26	2448	23	143	27
hsa-miR-4271	FAM25A	140	-20.4	140	-20.4	2483	19	56	10
hsa-miR-3665	KISS1	176	-36.81	176	-36.81	2630	18	143	123
hsa-miR-3180	KISS1	282	-46.02	142	-25.22	2706	19	143	68 112
hsa-miR-4463	FAM25A	163	-34.5	163	-34.5	2951	17	56	17
hsa-miR-4466	KISS1	156	-25.99	156	-25.99	2964	18	143	120
hsa-miR-4488	KISS1	296	-51.09	156	-30.08	3028	18	143	70 37
hsa-miR-3960	KISS1	141	-42.59	141	-42.59	3156	20	143	69
hsa-miR-4649-5p	KISS1	142	-31.55	142	-31.55	3220	24	143	62
hsa-miR-4651	KISS1	286	-62.95	144	-32.75	3228	20	143	61 73
hsa-miR-4653-3p	KISS1	140	-21.24	140	-21.24	3236	23	143	85
hsa-miR-4656	KISS1	158	-35.67	158	-35.67	3244	23	143	43
hsa-miR-4667-5p	FAM25A	143	-20.76	143	-20.76	3283	22	56	6
hsa-miR-4690-5p	KISS1	140	-25.99	140	-25.99	3358	22	143	78
hsa-miR-4697-5p	KISS1	293	-51.18	150	-27.45	3382	22	143	30 65
hsa-miR-4700-5p	FAM25A	156	-23.16	156	-23.16	3391	22	56	11
hsa-miR-4706	KISS1	151	-27.53	151	-27.53	3410	25	143	52
hsa-miR-4725-3p	FAM25A	153	-27.83	153	-27.83	3489	22	56	9
hsa-miR-4728-5p	FAM25A	149	-27.02	149	-27.02	3499	23	56	4
hsa-miR-4728-5p	KISS1	149	-24.92	149	-24.92	3500	23	143	35
hsa-miR-4734	KISS1	144	-28.92	144	-28.92	3520	22	143	75
hsa-miR-4739	KISS1	166	-33.97	166	-33.97	3538	25	143	34

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-4747-5p	FAM25A	155	-22.11	155	-22.11	3567	22	56	7
hsa-miR-4749-5p	KISS1	158	-28.21	158	-28.21	3574	22	143	52
hsa-miR-4763-3p	KISS1	140	-35.98	140	-35.98	3632	24	143	67
hsa-miR-4787-5p	KISS1	147	-35.92	147	-35.92	3718	22	143	66
hsa-miR-5088-5p	KISS1	140	-26.99	140	-26.99	3836	24	143	43
hsa-miR-5090	KISS1	148	-23.21	148	-23.21	3844	23	143	111
hsa-miR-5196-5p	FAM25A	150	-25.61	150	-25.61	3879	22	56	7
hsa-miR-5100	KISS1	151	-25.04	151	-25.04	3896	22	143	98
hsa-miR-664b-5p	KISS1	165	-31.07	165	-31.07	3916	24	143	42
hsa-miR-5698	FAM25A	148	-24.28	148	-24.28	4027	22	56	7
hsa-miR-5787	KISS1	142	-37.64	142	-37.64	4056	20	143	71
hsa-miR-6073	KISS1	142	-21.14	142	-21.14	4072	20	143	111
hsa-miR-6086	FAM25A	141	-28.17	141	-28.17	4097	20	56	14
hsa-miR-6090	KISS1	145	-41.3	145	-41.3	4104	19	143	69
hsa-miR-6127	KISS1	144	-30.3	144	-30.3	4112	19	143	40
hsa-miR-6133	KISS1	140	-24.97	140	-24.97	4126	19	143	40
hsa-miR-6499-5p	KISS1	140	-21.39	140	-21.39	4132	22	143	124
hsa-miR-6500-5p	KISS1	141	-24.94	141	-24.94	4140	24	143	22
hsa-miR-6727-5p	KISS1	147	-33.54	147	-33.54	4248	23	143	73
hsa-miR-6752-5p	KISS1	292	-61.54	147	-32.47	4346	22	143	59 32
hsa-miR-6754-5p	KISS1	144	-26.64	144	-26.64	4354	22	143	39
hsa-miR-6756-5p	KISS1	160	-40.95	160	-40.95	4362	23	143	68
hsa-miR-6763-5p	FAM25A	149	-30.68	149	-30.68	4389	19	56	11

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-6775-5p	KISS1	292	-64.84	147	-42.3	4438	25	143	78 110
hsa-miR-6779-5p	KISS1	140	-27.54	140	-27.54	4454	21	143	35
hsa-miR-6780a-5p	KISS1	140	-30.15	140	-30.15	4458	23	143	40
hsa-miR-6785-5p	FAM25A	143	-29.58	143	-29.58	4477	22	56	4
hsa-miR-6785-5p	KISS1	149	-23.04	149	-23.04	4478	22	143	37
hsa-miR-6787-5p	KISS1	313	-52.91	161	-26.82	4486	22	143	54 71
hsa-miR-6796-5p	KISS1	140	-22.78	140	-22.78	4522	23	143	73
hsa-miR-6803-5p	KISS1	155	-44.31	155	-44.31	4550	22	143	67
hsa-miR-6805-5p	KISS1	150	-25.79	150	-25.79	4558	22	143	35
hsa-miR-6810-5p	KISS1	140	-23.43	140	-23.43	4578	23	143	51
hsa-miR-6813-5p	FAM25A	144	-26.88	144	-26.88	4589	23	56	2
hsa-miR-6814-5p	KISS1	140	-23.38	140	-23.38	4594	22	143	90
hsa-miR-6816-5p	KISS1	307	-55.38	154	-29.08	4602	21	143	72 60
hsa-miR-6824-5p	KISS1	144	-26.23	144	-26.23	4634	22	143	41
hsa-miR-6825-5p	FAM25A	142	-26.35	142	-26.35	4637	22	56	4
hsa-miR-6780b-5p	FAM25A	156	-29.83	156	-29.83	4681	23	56	4
hsa-miR-6860	FAM25A	146	-35.29	146	-35.29	4781	22	56	8
hsa-miR-6874-5p	KISS1	152	-24.31	152	-24.31	4834	23	143	23
hsa-miR-6883-5p	FAM25A	146	-26.98	146	-26.98	4869	22	56	4
hsa-miR-6883-5p	KISS1	152	-27.09	152	-27.09	4870	22	143	37
hsa-miR-6884-5p	FAM25A	144	-20.72	144	-20.72	4873	22	56	13

Seq1	Seq2	Tot Score	Tot Energy	Max Score	Max Energy	Strand	Len1	Len2	Positions
hsa-miR-6885-5p	KISS1	289	-47.02	149	-25.56	4878	25	143	55 30
hsa-miR-7109-5p	KISS1	143	-22.81	143	-22.81	4934	21	143	62
hsa-miR-7110-5p	KISS1	145	-29.07	145	-29.07	4938	21	143	67
hsa-miR-8072	KISS1	145	-35.26	145	-35.26	5116	20	143	60
hsa-miR-8089	FAM25A	159	-30.91	159	-30.91	5149	24	56	7
hsa-miR-10394-3p	KISS1	144	-29.43	144	-29.43	5194	23	143	119
hsa-miR-10396a-5p	KISS1	294	-51.05	147	-28.57	5200	19	143	77 116
hsa-miR-10396b-5p	KISS1	158	-25.72	158	-25.72	5224	20	143	65

Construction of ceRNA network

Based on the interaction pairs of lncRNA-miRNA, mRNA-miRNA, and lncRNA-mRNA, a ceRNA network was constructed with 11 miRNAs, one lncRNA, and one mRNA, including SNHG24-has-miR-4739-KISS1, SNHG24-has-miR-4734-KISS1, and SNHG24-has-miR-135b-3p-KISS1 (Fig. 8). These RNA interactions may contribute to the progression and metastasis of pancreatic cancer.

GO functional and pathway enrichment analysis in ceRNA network

Based on the lncRNA-mRNA interaction pairs in the ceRNA network, KEGG pathway annotation was performed. The results revealed that SNHG24 mainly correlated with GnRH secretion and neuroactive ligand-receptor interaction in pancreatic cancer (Fig. 9).

Discussion

Pancreatic cancer is the most malignant tumor of the digestive system, and it is also known as the 'king of cancers' [24]. According to the latest statistical data, the incidence of pancreatic cancer ranks eighth among all malignant tumors in China, and the cumulative 5-year survival rate is only 7.2% [25]. Although growing number of studies have focused on dissecting and evaluating the underlying molecular etiology of pancreatic cancer over the past few years, the precise mechanisms of pancreatic cancer initiation and development are still poorly understood. In our study, eight DEsnoRNAs and 93 trend-related snoRNAs were identified. Ten host genes of snoRNAs were also identified, and functional analyses revealed that the ten host genes were significantly enriched in GO terms including mitotic chromosome condensation and endocytosis pathway. SNORA38B was found to be associated with survival and prognosis. SNORD17 and SNORA11 were found to be negatively correlated with methylation. Two trend-related SNHGs were also extracted. Additionally, a ceRNA network was constructed with the 11 miRNAs, one lncRNAs, and one mRNA. SNHG24 was mainly correlated with GnRH secretion and neuroactive ligand-receptor interaction in pancreatic cancer.

SNORD17, also known as HBI-43, has been shown to be involved in the differentiation of different cancer subtypes [26]. A recent report showed that SNORD17 was functionally related to a specific active gene TRIM25 [27]. Wang et al. [28] demonstrated that the control of TRIM25 RNA by an interplay between IGF2BP3 and miR-3614-3p represents a mechanism for breast cancer cell proliferation. More notably, Li et al. [29] reported that suppression of TRIM25 expression using high levels of miR-873 dictates MTA1 protein upregulation in hepatocellular carcinoma. Therefore, it is quite reasonable to speculate that as the regulator of TRIM25, the expression pattern of SNORD17 may also be sensitive to distinguish the different cancer subtypes. Interestingly, Davanian et al. [30] first identified SNORA11 as a distinct ncRNA signature of ameloblastoma. Herein, our methylation correlation analysis showed that SNORD17 and SNORA11 were also negatively correlated with methylation. Taken together, our results suggest that SNORD17 and SNORA11 are likely associated with pancreatic cancer occurrence and progression. In addition, our functional analysis showed that SNORD17 participated in some BPs, especially in endocytosis. Numerous studies have reported that endocytosis serves as a key regulatory mechanism in carcinogenesis [31, 32], which further implies that the molecular interactions between SNORD17 and endocytosis may be a novel therapeutic target for pancreatic cancer treatment. In this study, we also found SNORA38B to be associated with survival and prognosis of pancreatic cancer. Until now, SNORA38B has not been associated with any other neoplasms, thus making it unique in this respect.

C1orf79, also known as SNHG12, is a novel lncRNA identified to be upregulated in several cancer cells, such as human osteosarcoma cell [33], nasopharyngeal carcinoma cell [34], and human endometrial carcinoma [35]. Zhao et al. [36] verified that SNHG12 promotes angiogenesis after ischemic stroke through the miR-150/VEGF pathway, that further clarified the mechanism of angiogenesis after ischemic stroke, and provided a target for the treatment of ischemic stroke. Wang et al. [37] suggested that SNHG12 contributes to the oncogenic potential of triple-negative breast cancer and may be a promising therapeutic target. Wang et al. [38] illustrated that SNHG12 promoted cell growth and inhibited cell apoptosis in CRC cells, indicating that SNHG12 might be a useful biomarker for colorectal cancer. In the current study, *C1orf79* was identified as the host gene of DEsnoRNAs and trend-related snoRNAs. *C1orf79* is a new biomarker that to the best of our knowledge has not yet been reported to be associated with pancreatic cancer.

SNHG24, a prominent regulator of the ceRNA network, was found to be closely linked with the process of GnRH secretion and neuroactive ligand-receptor interaction, and exhibited significant correlation with KISS1. Reports suggest that the lncRNA SNHG24 can regulate TNF- α expression, and TNF- α and its associated lncRNA SNHG24 represent potential therapeutic targets for bone-invasive pituitary adenomas in the future [39]. Eckstein et al. [40] highlighted a strong correlation between GnRH secretion and the risk of prostate cancer, which directly supports our findings. Reports suggest that the pathway of neuroactive ligand-receptor interaction is associated with a variety of cancers [41], which further implies that the molecular interactions between SNHG24, GnRH secretion, and neuroactive ligand-receptor interaction may be a novel therapeutic target for pancreatic cancer treatment. In addition, Wang et al. [42] showed that an increase in the expression of KISS1 could inhibit invasion of pancreatic cancer cells without affecting cell proliferation. McNally et al. [43] suggested that the induction of KISS1 expression has the potential of adjuvant therapy in pancreatic cancer. More interestingly, the ceRNA-related with SNHG24-KISS1 network analysis found that SNHG24-KISS1 had a strong relationship with miRNAs such as has-miR-4739, has-miR-4734, and has-miR-135b-3p. Increasing evidence suggest that miRNA serve as functional modulators in tumor development [44, 45]. Taken together, we speculate that miRNAs such as has-miR-4739, has-miR-4734, and has-miR-135b-3p contribute to pancreatic cancer progression possibly by targeting the SNHG24-KISS1 axis, which still needs to be confirmed through further bioinformatics analysis and experimental evidence.

Although we explored the potential molecular mechanisms of pancreatic cancer occurrence and development using a bioinformatics approach, there were still several limitations in this study. Firstly, no DESNHGs were obtained in this study. In addition, relevant experiments including cell biology assays, and animal and clinical studies need to be performed to verify the multiple candidate targets and signaling pathways identified from our bioinformatics analyses.

Conclusion

The snoRNAs and SNHGs identified in our study may serve as potential markers for the early detection of pancreatic cancer. However, our findings still need to be validated through further studies.

Abbreviations

Abbreviation	Full name
snoRNAs	Small nucleolar noncoding RNAs
SNHG	Small nucleolar RNA host gene
DEsnoRNAs	Differentially expressed snoRNAs
DESNHGs	Differentially expressed SNHGs
TCGA	The cancer genome atlas
KEGG	Kyoto Encyclopedia of Genes and Genomes
GLOBOCAN	Global cancer epidemic statistics
lncRNAs	Long noncoding RNAs
ceRNAs	Competing endogenous RNAs
BP	Biological process
CC	Cellular components
MF	Molecular function
GO	Gene ontology

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not Applicable.

Availability of data and materials

The datasets generated during the current study are not publicly available but obtained from corresponding authors on reasonable request.

Competing Interests

The authors declare that no conflicts of interest exist.

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Authors' Contributions

All authors participated in the conception and design of the study;

Conceived and drafted the manuscript: Shuwen Han and Xi Yang ;

Analyzed the data: Siqi Dai ,Yangyang Xie , Xi Yang and Xiaoyu Dai ;

Wrote the paper:Shuwen Han and Yangyang Xie;

All authors read and approved the paper.

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References

- [1] Ilic M, Ilic I (2016). Epidemiology of pancreatic cancer. *World J Gastroenterol*, 22:9694-9705.
- [2] McGuire S (2016). *World Cancer Report 2014*. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. *Advances in Nutrition*, 7:418-419.
- [3] Bray F, Ferlay J, Soerjomataram I, et al (2018). Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*, 68:394-424.
- [4] Conroy T, Van Laethem J-L (2019). Combination or single-agent chemotherapy as adjuvant treatment for pancreatic cancer? *The Lancet Oncology*.
- [5] Boeck S, Heinemann V Improving post-surgical management of resected pancreatic cancer. *Lancet*, 390:847-848.
- [6] Are C, Chowdhury S, Ahmad H, Ravipati A, Song T, Shrikandhe S, et al. Predictive global trends in the incidence and mortality of pancreatic cancer based on geographic location, socio-economic status, and demographic shift. *Journal of Surgical Oncology*.
- [7] Xu B, Liu J, Xiang X, Liu S, Zhong P, Xie F, et al. (2018). Expression of miRNA-143 in Pancreatic Cancer and Its Clinical Significance. *Cancer Biother Radiopharm*, 33:373-379.

- [8] Li X, Deng SJ, Zhu S, Jin Y, Cui SP, Chen JY, *et al.* (2016). Hypoxia-induced lncRNA-NUTF2P3-001 contributes to tumorigenesis of pancreatic cancer by derepressing the miR-3923/KRAS pathway. *Oncotarget*, 7:6000-6014.
- [9] Qadir MI, Faheem A (2017). miRNA: A Diagnostic and Therapeutic Tool for Pancreatic Cancer. *Crit Rev Eukaryot Gene Expr*, 27:197-204.
- [10] Bhan A, Soleimani M, Mandal SS (2017). Long Noncoding RNA and Cancer: A New Paradigm. *Cancer Res*, 77:3965-3981.
- [11] Fu Z, Chen C, Zhou Q, Wang Y, Zhao Y, Zhao X, *et al.* LncRNA HOTTIP modulates cancer stem cell properties in human pancreatic cancer by regulating HOXA9. *Cancer Letters*:S0304383517305694.
- [12] Cheng D, Fan J, Ma Y, Zhou Y, Qin K, Shi M, *et al.* LncRNA SNHG7 promotes pancreatic cancer proliferation through ID4 by sponging miR-342-3p. *Cell & Bioscience*, 9.
- [13] Dong XY, Guo P, Boyd J, Sun X, Li Q, Zhou W, *et al.* (2009). Implication of snoRNA U50 in human breast cancer. *J Genet Genomics*, 36:447-454.
- [14] Okugawa Y, Toiyama Y, Toden S, Mitoma H, Nagasaka T, Tanaka K, *et al.* (2017). Clinical significance of SNORA42 as an oncogene and a prognostic biomarker in colorectal cancer. *Gut*, 66:107-117.
- [15] Popov A, Szabo A, Mandys V Small nucleolar RNA U91 is a new internal control for accurate microRNAs quantification in pancreatic cancer. *Bmc Cancer*, 15:774.
- [16] Goldman M, Craft B, Zhu J, Haussler D (2019). The UCSC Xena system for cancer genomics data visualization and interpretation. *Cancer Research*, 77:326470.
- [17] MD R, DJ M, GK S (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 26:139-140.
- [18] Ritchie ME, Belinda P, Di W, Hu Y, Law CW, Wei S, *et al.* (2015). \n limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*:7.
- [19] Ernst J, Bar-Joseph Z STEM: a tool for the analysis of short time series gene expression data. 7:191-190.
- [20] Yoshihama M, Nakao A, Kenmochi N (2013). snOPY: a small nucleolar RNA orthological gene database. *Bmc Research Notes*, 6:1-5.
- [21] Yu G, Wang L-G, Han Y, He Q-Y clusterProfiler: an R Package for Comparing Biological Themes Among Gene Clusters. *OMICS-A JOURNAL OF INTEGRATIVE BIOLOGY*, 16:284-287.
- [22] Gong J, Li Y, Liu C-j, Xiang Y, Li C, Ye Y, *et al.* A Pan-cancer Analysis of the Expression and Clinical Relevance of Small Nucleolar RNAs in Human Cancer. *Cell Reports*, 21:1968-1981.
- [23] Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS (2003). MicroRNA targets in *Drosophila*. *Genome Biol*, 5:R1.
- [24] Timaner M, Shaked Y (2019). Elucidating the roles of ASPM isoforms reveals a novel prognostic marker for pancreatic cancer. *The Journal of Pathology*.

- [25] Zeng H, Chen W, R Z (2018). Changing cancer survival in China during 2003–15: a pooled analysis of 17 population-based cancer registries. *Lancet Global Health*, 6:e555-e567.
- [26] Pan X, Chen L (2019). Analysis of Expression Pattern of snoRNAs in Different Cancer Types with Machine Learning Algorithms. 20.
- [27] Choudhury NR, Heikel G, Trubitsyna M, Kubik P, Nowak JS, Webb S, *et al.* (2017). RNA-binding activity of TRIM25 is mediated by its PRY/SPRY domain and is required for ubiquitination. 15:105.
- [28] Wang Z, Tong D, Han C, Zhao Z, Wang X, Jiang T, *et al.* (2019). Blockade of miR-3614 maturation by IGF2BP3 increases TRIM25 expression and promotes breast cancer cell proliferation. *EBioMedicine*, 41:357-369.
- [29] Li YH, Zhong M, Zang HL, Tian XF (2018). The E3 ligase for metastasis associated 1 protein, TRIM25, is targeted by microRNA-873 in hepatocellular carcinoma. *Exp Cell Res*, 368:37-41.
- [30] Davanian H, Balasiddaiah A, Heymann R, Sundstrom M, Redenstrom P, Silfverberg M, *et al.* (2017). Ameloblastoma RNA profiling uncovers a distinct non-coding RNA signature. *Oncotarget*, 8:4530-4542.
- [31] Zhang Y, Zhang B (2008). TRAIL resistance of breast cancer cells is associated with constitutive endocytosis of death receptors 4 and 5. *Mol Cancer Res*, 6:1861-1871.
- [32] Yeager A (2013). Investigation of caveolae-mediated endocytosis in response to interferon.
- [33] Ruan W, Wang P, Feng S, Xue Y, Li Y (2016). Long non-coding RNA small nucleolar RNA host gene 12 (SNHG12) promotes cell proliferation and migration by upregulating angiomin gene expression in human osteosarcoma cells. *Tumour Biol*, 37:4065-4073.
- [34] Gong Z, Zhang S, Zeng Z, Wu H, Yang Q, Xiong F, *et al.* (2014). LOC401317, a p53-regulated long non-coding RNA, inhibits cell proliferation and induces apoptosis in the nasopharyngeal carcinoma cell line HNE2. *PLoS One*, 9:e110674.
- [35] Zhai W, Li X, Wu S, Zhang Y, Pang H, Chen W (2015). Microarray expression profile of lncRNAs and the upregulated ASLNC04080 lncRNA in human endometrial carcinoma. *Int J Oncol*, 46:2125-2137.
- [36] Zhao M, Wang J, Xi X, Tan N, Zhang L (2018). SNHG12 Promotes Angiogenesis Following Ischemic Stroke via Regulating miR-150/VEGF Pathway. *Neuroscience*, 390:231-240.
- [37] Wang O, Yang F, Liu Y, Lv L, Ma R, Chen C, *et al.* (2017). C-MYC-induced upregulation of lncRNA SNHG12 regulates cell proliferation, apoptosis and migration in triple-negative breast cancer. *Am J Transl Res*, 9:533-545.
- [38] Wang JZ, Xu CL, Wu H, Shen SJ (2017). LncRNA SNHG12 promotes cell growth and inhibits cell apoptosis in colorectal cancer cells. *Braz J Med Biol Res*, 50:e6079.
- [39] Zhu H, Guo J, Shen Y, Dong W, Gao H, Miao Y, *et al.* (2018). Functions and Mechanisms of Tumor Necrosis Factor- α and Noncoding RNAs in Bone-Invasive Pituitary Adenomas. *Clin Cancer Res*, 24:5757-5766.
- [40] Eckstein N, Haas B Clinical pharmacology and regulatory consequences of GnRH analogues in prostate cancer. *European Journal of Clinical Pharmacology*, 70:791-798.

[41] Yang Z, Yan Z, Chen L. 2012. in silico identification of novel cancer-related genes by comparative genomics of naked mole rat and rat.

[42] Wang CH, Qiao C, Wang RC, Zhou WP (2016). KiSS1 mediated suppression of the invasive ability of human pancreatic carcinoma cells is not dependent on the level of KiSS1 receptor GPR54. *Mol Med Rep*, 13:123-129.

[43] McNally LR, Welch DR, Beck BH, Stafford LJ, Long JW, Sellers JC, *et al.* (2010). KISS1 over-expression suppresses metastasis of pancreatic adenocarcinoma in a xenograft mouse model. *Clin Exp Metastasis*, 27:591-600.

[44] Rathod SS, Rani SB, Khan M, Muzumdar D, Shiras A Tumor suppressive miRNA-34a suppresses cell proliferation and tumor growth of glioma stem cells by targeting Akt and Wnt signaling pathways. *Febs Open Bio*, 4:485-495.

[45] Fang RH, Ji XB (2018). [Advances in the research of the relationship between miRNA-29c and cancer]. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*, 32:312-317.

Figures

Figure 1

The flow chart of this study

Figure 2

Identification of differentially expressed snoRNAs (DEsnoRNAs) and trend-related snoRNAs To screen the differentially expressed snoRNAs (DEsnoRNAs) between the tumor and normal groups based on the read count data of the snoRNAs, the TMM (trimmed mean of m values) normalization provided by the R software package edge was used to preprocess the read count. Then the quasi-likelihood (QL) F-test in the edgeR package was applied for screening the DEsnoRNAs, and the screening criteria was $p < 0.05$, $|\log FC| > 1$ (foldchange > 1.2). A total of eight DEsnoRNAs, including two upregulated and six downregulated snoRNAs were identified. A: Heatmap of DEsnoRNAs; B: volcano plot of DEsnoRNAs

Figure 3

Identification of trend-related snoRNAs The trend-related snoRNAs were obtained based on the classification of tumor stage diagnosis, including stage I, stage II, stage II, and stage IV. The data was analyzed using the STEM software for trend analysis. The processing settings included with 'No normalization/add 0' and screened with $p < 0.05$. A total of 93 trend-related snoRNAs were obtained. A: Sample diagram of trend analysis. The colored module in the figure represents significant trends with $p < 0.05$. Each colored line represents the expression trend of a gene at different points with the black line representing the general trend of the changes of these genes. The colorless modules represent non-significant trends, and were excluded from the subsequent analyses. B: Sample diagram of a module. A single module in the sample graph of the trend analysis is magnified to show the expression of each gene according to the stage node.

Figure 4

Functional analysis of host gene of DESnoRNAs and trend-related snoRNAs To functionally analyze the host gene of DESnoRNAs and trend-related snoRNAs, based on the target genes of the snoRNAs, the clusterprofiler tool was used to perform GO functional and KEGG pathway analyses. The functional analyses indicated that host genes of DESnoRNAs were significantly enriched in several GO terms including mitotic chromosome condensation, retromer complex, and dopamine receptor binding, and endocytosis pathway, while host gene of trend-related snoRNAs were predominantly enriched in mitotic chromosome condensation, presynaptic active zone, and sphingolipid binding. A: The Gene ontology (GO) functional analysis of host gene of DESnoRNAs; B: The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of host gene of DESnoRNAs; C: The Gene ontology (GO) functional analysis of top 10 host gene of trend-related snoRNAs.

Figure 5

Survival analysis of DEsnRNAs and trend-related snoRNAs To evaluate the association of the snoRNAs with survival and prognosis, the survival analysis is conducted on the DEsnRNAs and trend-related snoRNAs data. $p < 0.05$ was considered to be an association with survival and prognosis. SNORA38B, a DEsnRNA with $p = 0.0092$, was found to be associated with survival and prognosis. A: SCARNA10; B: SNORA38B; C: SNORA44; D: SNORA54; E: SNORA73B; F: SNORD15B; G: SNORD17; H: SNORD53_SNORD92; I: ACA59; J: SCARNA10; K: SCARNA12; L: SNORA11; M: SNORA12; N: SNORA13; O: SNORA14B; P: SNORA16B; Q: SNORA16

Figure 6

Methylation correlation analysis of DEsnoRNAs and trend-related snoRNAs To evaluate the association of the snoRNAs with methylation, methylation correlation analysis was performed using the SNORic tool. SNORD17 and SNORA11 were found to be negatively correlated with methylation. A: The methylation correlation analysis of DEsnoRNAs; B: The methylation correlation analysis of trend-related snoRNAs. The abscissa shows the expression value of the snoRNA sample, the ordinate shows the expression value of the methylated sample, and the blue line is the trend line. In general, a positive correlation has a positive coefficient, and a negative correlation has a negative coefficient. When the correlation coefficient is relatively large, the correlation is relatively strong and the points are more concentrated on both sides of the trend line.

Figure 7

Identification of trend-related SNHG2s To obtain the trend-related SNHG2s based on the classification of tumor stage diagnosis, including stage I, stage II, stage III, and stage IV, the data was processed using STEM software for trend analysis. First, the data was processed with 'No normalization/add 0' and screened with $p < 0.05$. Then the data was processed according to the parameter 'normalize data' and screened with $p < 0.1$. According to the single module, there was no obvious change trend among the various stages. Following amplification, the expression of each gene is shown according to the stage node. Two SNHG2s, including SNHG23 and SNHG24, have obvious trend changes. A: The trend analysis of 'No normalization/add 0'. The colored module in the figure represents significant trend with $p < 0.05$. Each colored line represents the expression trend of a gene at different points, and the black line represents the general trend of the change of these genes. The colorless modules represent non-significant trends and were excluded from the subsequent analyses. B: The trend analysis of 'normalize' module with $p < 0.05$. There are no colored modules. C: The results of 'No normalize/add 0' module. A single module in the sample graph of trend analysis is magnified to show the expression of each gene according to the stage node. D: The results of 'normalize' single module.

Figure 8

Construction of ceRNA network To identify the ceRNA interaction pairs, the interaction pairs of lncRNA-mRNA, lncRNA-miRNA, and mRNA-miRNA were integrated to construct the ceRNA network. The ceRNA network was constructed with 11 miRNAs, one lncRNA and one mRNA. The red circle represents SNHG2s, the green circle represents mRNA, and the blue circle represents miRNA.

Figure 9

Gene ontology (GO) functional and pathway enrichment analysis in ceRNA network To identify the pathways that might be affected by the SNHG24, the genes in the ceRNA network were subjected to KEGG pathway annotation using the clusterprofiler tool. The results revealed that SNHG24 is mainly correlated with GnRH secretion and neuroactive ligand-receptor interaction in pancreatic cancer.

Supplementary Files

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