Genome-wide Long Noncoding RNA and mRNA Expression Profile Reveals Associations Between Inorganic Elements Exposure and Risk of Developing Hepatocellular Carcinoma

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Abstract

Background The long non-coding RNAs (lncRNA) have been closely associated with the development of hepatocellular carcinoma (HCC). The present study conducted a genome-wide microarray analysis and qPCR validation to gain a comprehensive insight on this issue. Methods Thirty male HCC patients with chronic HBV infection were included in the present study. Primary HCC tissues and normal tissue were collected. Synthesize double stranded complementary DNA from 10 pairs of samples were labeled and hybridized to microarray chip. Further analysis, such as hierarchical clustering analysis, gene ontology (GO) and pathway analysis were performed. In addition, qPCR validation was conducted in all samples. Results The microarray analysis identified 946 upregulated and 571 downregulated lncRNAs, along with 1,720 upregulated and 1,106 downregulated mRNAs. Among them, ENST00000583827.1 (fold change: 21.11) and uc010isf.1 (fold change: 17.76) were the most over- and under-expressed lncRNAs in HCC tissues. As for the mRNAs, KIF20A (fold change: 26.44) and HEPACAM (fold change: 49.56) were the most over- and under-expressed in HCC tissues. GO analysis revealed that the most differentially expressed mRNAs were related with the response of metal ion. Pathway analysis also suggested the most enriched pathway was mineral absorption. Conclusion The subsequent qPCR validation exhibited high consistency with microarray analysis, expect one lncRNA. It also revealed that TCONS_00008984 had 768.94-fold expression level in HCC tissues when compared with normal tissues. TCONS_00008984 has the potential to serve as a diagnostic marker or prognosis indicator. GO and pathway analysis that exposure to inorganic elements may possibly involve with HCC risk.

Background

Primary hepatocellular carcinoma (HCC) has been qualified as the most common malignancy of liver in worldwide range and the high fatality it caused has placed heavy burden on both patients and healthcare system. Annually, half a million incidences of HCC emerge and 1% of total deaths are closely related with HCC worldwide. With the progress of medical science, presently the chronic infection of hepatitis B virus and hepatitis C virus, along with the potential cirrhosis and fibrosis, have been acknowledged as the major risk factor of developing HCC and accounted for approximately 80% of HCC incidence [1–2]. Based on evidence acquired previously, it can be concluded that most regions with high prevalence of HCC are overlapped with high prevalence of HBV or HCV infection. To be more precise, among these hepatitis viruses related HCC patients, HBV is responsible for 75–80% of virus-related HCC cases, while HCV accounts for remaining 10–20%[3]. China has the largest population with chronic HBV infection in the world, with the effort made in immunization program and management of chronic HBV patients, the prevalence rate of HBV dropped from 9.8–7.2% in general population[4]. However, due to the absence of ideal therapy to eradicate the chronic HBV infection, still a large population suffered from the chronic HBV infection and its possible complications, and this specific population is at high risk of developing HCC. Owing to this circumstance, globally about half of HCC cases are reported in China which has been recognized as a pressing public health issue[5].

As revealed by statistics, male has approximately 4-fold risk of developing HCC when comparing with their female counterparts, the reason behind these phenomena is that males have higher chances to be chronically infected with hepatitis viruses considering their transmission route, alcohol consumption, smoking, and elevated iron level. Even within the population with chronic infection of viral hepatitis, men still maintain higher incidence than women, reports also observed higher levels of aflatoxin markers in blood samples collected from male subjects when comparing with female[6]. Therefore, the male with chronic HBV infection in China definitely can

be identified as the high risk population of developing HCC, and the etiology study of HCC targeted specifically in this population is urgently needed for providing scientific evidence for the intervention and prevention.

When being compared with protein coding region, the non-coding region is consisted approximately 97.2% of the whole genome of human[7], in the beginning, the non-coding region was considered as non-functional and waste of transcripts[8]. However, due to the rapid development of high-throughput sequencing method and microarray technology, a huge amount of non-coding RNAs including long non-coding RNAs (lncRNAs) have been identified in mammalian cells. Up to date, a wide variety of biological functions of lncRNAs have been discovered, including X chromosome inactivation[9], regulation of gene expression[10], and more importantly, have direct impact on mRNA stability[11]. A number of investigations have been conducted to study the association between lncRNAs and the risk of developing HCC, among them, the identification of lncRNA highly-up-regulated in liver cancer (HULC) was the most typical finding. HULC is one of the most excessively expressed lncRNAs in HCC tissue, and it is capable of suppressing several microRNAs (miRNAs), including miR-372, and consequently result in an auto-regulatory loop in which HULC promotes its own expression[12]. Moreover, the expression of HULC is associated with hepatitis B virus X protein (HBx) in HCC tissue, and inversely correlated with tumor suppressor gene p18 located near HULC in the same chromosome[13]. Therefore, it can be concluded that the expression of HULC involved with the proliferation of hepatoma cells and the disease progression. Although certain evidences have been obtained by previous investigators, individual studies were mostly focused on some particular lncRNAs, and microarray analysis which can cover all known lncRNA was conducted in a limited sample size due to the expansive cost of employing high-throughput lncRNA chip. More importantly, the previous studies were not focused on the male HCC patients with chronic HBV infection, and apparently, failed to investigate the impact of HBV infection on the lncRNA expression profile. In order to further expand the statistical power of such approaches and to gain a comprehensive insight of lncRNA expression profile in male HCC patients with chronic HBV infection, we conducted a microarray analysis in 20 paired HCC tissues and adjacent tissues, and qPCR validation was conducted among 60 paired tissues to further support the results generated from microarray analysis.

Materials And Methods

Study participant and sample preparation

During the study period of November 2015 to December 2017, 30 male HCC patients with chronic HBV infection was included in the present study. The inclusion criteria were as follows: (1) pathologically diagnosed with primary HCC (ICD9-155); (2) male; (3) chronic HBV infection confirmed by ELISA prior to the onset of HCC; (4) permanent residents who lived in Xiamen over 10 years and aging from 20 to 79 years. Patients were excluded if any of the following conditions were met:(1) liver disease due to parasitosis, diabetes, fatty liver, metabolism disorders or severe cardiovascular diseases; (2) presence of cancers other than HCC; (3) autoimmune hepatitis or toxic hepatitis; (4) refuse to participate. Primary HCC tissues and normal tissue distant from tumor edge for 5 cm were collected from each included patients during the liver resection and placed in liquid nitrogen pre-freezing RNase-free vial for 5 min, and stored at -78 °C prior to RNA extraction. Tissue samples were subjected to RNA extraction using Trizol reagent (Invitrogen, MA, USA). The purity and concentration of RNA were determined from OD260/280 readings using a NanoDrop ND-1000, and the integrity was evaluated using standard denaturing agarose gel electrophoresis. RNA extracts with total volume exceeded 8 μg undergone further analysis. The present study was approved by the Ethical Committee of Zhongshan Hospital, Xiamen University after formal

hearing, and in conforms with the Declaration of Helsinki. The written consent has been presented to every study participant for reviewing before their operation. In the consent, the detailed purpose of this study, the sample collection procedure, possible risk, confidentiality of patients' personal information, and the right to withdraw in any stage were clearly elaborated. All patients provided physically signed the written consent before sample collection.

Microarray analysis

In total, paired cancer and normal samples from 10 study participants were used to synthesize double stranded complementary DNA (cDNA), and the cDNA product was labeled and hybridized to lncRNA + mRNA Human Gene Expression Microarray V4.0 (CapitalBio Corp, Beijing, China) in accordance with the manufacturer's instructions. The microarray chip employed in the present study covers 40,916 human lncRNAs probes, 34,235 mRNA probes, and 4,974 Agilent control probes. Each RNA was detected by corresponding probes repeated for two times.

Microarray imaging and data analysis

Data summarization, normalization and quality control were conducted in the data generated from lncRNA + mRNA microarray by using GeneSpring software version 12.0 (Agilent, CA, USA). In order to identify the differentially expressed genes, we employed threshold values of $\geq$ 2-fold change and a Benjamini-Hochberg corrected *P* value of $\leq$ 0.05. The data was log 2 transformed and median centered by genes using Adjust Data function of Multiexperiment Viewer software (Dana-Farber Cancer Institute, MA, USA). Further analysis, such as hierarchical clustering with average linkages was performed. Treeview software (Stanford University, CA, USA) composed by Java was employed to visualize the microarray results.

Gene Ontology (GO) and pathway analysis

GO analysis provides three structured networks of defined terms that describe gene product properties, including biological process, cellular component, and molecular function. GO term enrichment and pathway analysis based on the latest KEGG database were conducted in the differentially expressed mRNAs between HCC tissue and normal tissue. This analysis enabled us to identify the biological pathways for differentially expressed mRNAs in acquired samples.

qPCR validation

Based on the data generated from microarray analysis, we selected 18 significantly up-regulated and 4 down-regulated lncRNAs in accordance with the fold change and biological replicates to determine their expression level by using qPCR. We included the 20 paired samples which subjected to microarray analysis, and 40 paired samples collected from 20 remaining study participants. Briefly, total RNA was extracted from tissue using TRIzol reagent (Invitrogen, CA, USA), and agarose gel electrophoresis was employed to evaluate the integrity of RNA product. Reverse transcript was conducted by using FastQuant RT Kit with gDNase (Tiangen, Beijing, China) according to the manufacturer's protocol. PCR amplification was performed in a total volume of 10 μl PCR, including 5 μL of Power SYBR Green PCR Mare Mix (Applied Biosystems, CA, USA), 0.25μL of forward primer and reverse primer each, 4μL of nuclease-free water. The reaction was performed as follows: enzyme activation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 s, anneal and extension at 60 °C for 1 min, and melting curve analysis was performed in temperature between 60 °C to 95 °C. The comparative CT method with the formula for relative fold-change = $2^{-\Delta\Delta CT}$ was used to quantify the amplified transcripts.

Results

Differential expression of lncRNAs and mRNAs in HCC and normal tissues

As stated above in the method section, the microarray we employed in present study covers 40,916 human lncRNAs probes. Among all tested lncRNAs, lncRNAs with more than two-fold expression changes ($P < 0.05$) and at least three biological replicates were considered differentially expressed. Based on these criteria, the microarray analysis identified 946 upregulated and 571 downregulated lncRNAs. The top 30 differentially expressed lncRNAs were listed in Table 1, as can be seen, ENST00000583827.1 (fold change: 21.11) and uc010isf.1 (fold change: 17.76) were the most over- and under-expressed lncRNAs in HCC tissues. Among the 34,235 mRNAs expression level detected, we found 1,720 upregulated and 1,106 downregulated mRNAs in accordance with the same criteria applied in screening lncRNAs. The KIF20A (fold change:26.44) and HEPACAM (fold change: 49.56) were the most over- and under-expressed mRNAs in HCC tissues when compared with their normal counterparts. The scatter plot of differentially expressed lncRNAs and mRNAs was demonstrated in Fig. 1 and the volcano plot was showed in Fig. 2.

Table 1

The top 30 differentially expressed lncRNAs and mRNAs between HCC and normal tissues

Rank	lncRNA			mRNA		
	GeneSymbol	Regulation	Fold Change	GeneSymbol	Regulation	Fold Change
1	ENST00000583827.1	up	21.11	HEPACAM	down	49.56
2	uc010isf.1	down	17.76	CLEC4G	down	44.74
3	XR_109840.2	down	16.41	CXCL14	down	38.37
4	TCONS_00023687	down	15.08	COLEC10	down	35.43
5	ENST00000596540.1	up	12.01	SLC25A47	down	31.15
6	RNA147023|p0127_imsncRNA205	up	11.88	KIF20A	up	26.44
7	ENST00000521666.1	up	11.30	HHIP	down	26.17
8	ENST00000559280.1	up	10.96	CLEC1B	down	23.87
9	NR_027462.1	down	10.94	CYP2E1	down	23.85
10	ENST00000591629.1	up	10.92	TOP2A	up	22.89
11	ENST00000437781.1	up	10.75	PLIN2	down	22.72
12	ENST00000568523.1	down	10.56	THBS4	up	22.61
13	TCONS_00005551	down	10.26	MEP1A	up	22.53
14	ENST00000597260.1	down	10.05	CYP26A1	down	22.18
15	TCONS_00012039	up	9.96	SPP1	up	19.81
16	ENST00000597655.1	up	9.74	AKR1B10	up	19.44
17	TCONS_00008984	up	9.73	HPD	down	18.85
18	ENST00000451264.1	down	9.60	HEPN1	down	18.42
19	ENST00000560278.1	up	9.47	MELK	up	18.35
20	ENST00000608652.1	up	9.39	BUB1	up	17.82
21	ENST00000600679.1	up	8.98	FCN2	down	17.76
22	ENST00000609879.1	up	8.94	NEB	up	17.22
23	XR_158989.2	down	8.73	MKI67	up	17.19
24	ENST00000584351.1	up	8.62	CD5L	down	17.07
25	ENST00000432120.1	up	8.58	SPINK1	up	17.01
26	ENST00000415810.1	up	8.35	DEPDC1	up	16.93

Rank	lncRNA			mRNA		
	GeneSymbol	Regulation	Fold Change	GeneSymbol	Regulation	Fold Change
27	TCONS_00023870	down	8.19	HHIP	down	16.84
28	TCONS_00023860	down	8.19	BMP10	down	16.45
29	TCONS_00016038	up	8.00	MAGEA2B	up	16.43
30	ENST00000566797.1	down	7.91	CDKN3	up	15.93

A hierarchical clustering analysis was conducted to group lncRNAs and mRNAs based on the expression level of all determined RNAs in microarray analysis, allowing us to hypothesize the relationship among samples. The denodrogram in Fig. 3 revealed the relationships of the lncRNA (Fig. 3a) and mRNA (Fig. 3b) expression profiles between HCC tissues and normal tissues.

Gene ontology (GO) and pathway analysis results

GO analysis was conducted to gain insight into the potential functions of the differentially expressed host gene in the HCC tissues when comparing with normal counterparts. Differentially expressed mRNAs from the microarray analysis were classified into different functional categories including biological process, cellular component, and molecular function. The Fig. 4 listed the top 30 enriched GO terms, and as can be seen, most of enriched GO terms were classified as biological process. More importantly, the top 10 enriched GO terms were all involved with biological process, to be more precise, most of the top 10 enriched GO terms were related with the response of metal ion. Only three cellular components ranked the top 30, and the most enriched term in this category was perinuclear region of cytoplasm (GO:004008). As for molecular function, six of them were listed in top 30 enriched GO terms, among them, the most enriched GO term was nerve growth factor binding (GO: 0048406).

Pathway analysis was used to investigate the involved biological pathways of the differentially expressed mRNAs in HCC carcinogenesis. The analysis suggested that the differentially expressed mRNAs in HCC tissue were mainly participated in mineral absorption (hsa04978), signaling by NIDAL (REACT_111057), signaling by NGF(REACT_11061), development biology (REACT_111045), calcium signal pathway (hsa04020) and signal transduction (REACT_111102). The detailed result of pathway analysis was showed in Fig. 5.

qPCR validation

The expression level of 18 significantly up-regulated and 4 down-regulated lncRNAs, which were selected based on the fold change and biological replicates, were determined by qPCR in 30 pairs of tissues collected from HCC patients with identical criteria of microarray analysis. The qPCR results were presented in Table 2 along with the results generated from microarray analysis of corresponding lncRNA. As can be seen, there was only one discrepancy between qPCR and microarray analysis. According to the microarray analysis, the expression level ENSG00000259889.1 was significantly elevated in HCC tissue when compared with normal tissue, however, the qPCR results demonstrated the ENSG00000259889.1 was down-regulated in HCC tissue, but the T-test showed the down-regulation in qPCR analysis was not significant ($P = 0.370$). The rest qPCR results of 21 lncRNA all

showed good alignment when comparing with microarray analysis, suggesting the solid property of microarray analysis of present study. What was striking about the qPCR results was that the TCONS_00008984 had 768.94-fold expression level in HCC tissue when compared with normal tissue. Moreover, the expression level of ENST00000591629.1 was elevated for 33.81 times in HCC tissue, according to qPCR results. The detailed comparison of microarray analysis and qPCR validation was demonstrated in Table 2 and Fig. 6. These highly altered lncRNAs could possibly involve with the carcinogenesis of HCC.

Table 2
The comparison on qPCR validation results of 22 lncRNAs and microarray data

lncRNA	Fold change	Regulation	Fold change	Regulation	*P* value
	Microarray	Microarray	qPCR validation	qPCR validation	
ENST00000585911.1	7.04	up	2.82	up	0.048
ENST00000591629.1	10.92	up	33.81	up	< 0.001
ENST00000415810.1	8.35	up	16.79	up	0.009
TCONS_00005551	10.26	down	14.83	down	< 0.001
ENST00000438128.1	3.14	up	12.55	up	0.001
ENST00000521666.1	11.30	up	5.07	up	< 0.001
TCONS_000016038	8.00	up	1.02	up	0.0069
ENST00000432120.1	8.58	up	6.49	up	< 0.001
ENST00000451368.1	2.54	down	15.78	down	< 0.001
TCONS_000012039	9.96	up	4.91	up	0.035
ENST00000583827.1	21.11	up	14.96	up	0.001
TCONS_00008984	9.73	up	768.94	up	0.019
ENSG00000269974.1	7.25	up	3.13	up	< 0.001
ENSG00000231969.1	7.20	down	2.21	down	< 0.001
ENSG00000267649.1	7.04	up	2.07	up	< 0.001
LOC100507474	6.70	up	2.37	up	< 0.001
XLOC_004430	6.65	up	3.27	up	0.004
ENSG00000226674.2	3.09	up	6.75	up	< 0.001
XLOC_003595	6.66	down	11.60	down	< 0.001
ENSG00000225210.5	7.65	up	10.94	up	< 0.001
ENSG00000259889.1*	1.23	up	2.19	Non-significant	0.370
ENSG00000244306.5	4.40	up	7.79	up	< 0.001
*Contradicted results between microarray analysis and qPCR validation					

Discussion

In recent years, the dysregulation of lncRNA expression profile has been studied widely to uncover the underlying molecular mechanism contributing to the development of human cancers[14]. With the rapid development on the microarray technology and sustaining increase on the coverage of human lncRNA, certain number of lncRNAs

have been found to be associated with the carcinogenesis. Microarray technology possesses several advantages, including high-throughput property covering whole human genome, high sensitivity, and capable of providing lncRNA-mRNA correlation analysis[15]. However, due to the expansive price of employing microarray chip, the studies often conducted in a limited sample size. Under such condition, the credibility of lncRNA may affected by random error or outlier. Therefore, it is imperative to conduct qPCR validation on larger sample size to verify the results generated by microarray analysis. In present study, we first investigated the lncRNA and mRNA expression profile in 10 HCC tissue and normal tissue collected from 10 male HCC patients with chronic HBV infection. Compared with the normal tissue, we identified 946 upregulated and 571 downregulated lncRNAs. As for mRNAs, 1,720 upregulated and 1,106 downregulated mRNAs were found by same criteria. Furthermore, qPCR was performed in 30 pairs of tissues to validate the expression level of 22 selected lncRNAs. Overall, the results between different methods showed high consistency except one lncRNA, suggesting the solid performance of microarray analysis.

We conducted the GO enrichment analysis to investigate the potential function and pathway of differentially expressed mRNAs. Surprisingly, the enriched GO terms were mostly involved with the metal ion response, including zinc and cadmium. The results supported evidence from the study conducted by Ebara et al, which observed accumulation of cooper-metallothionein (Cu-MT) in HCC tissue while the zinc metallothionein(Zn-MT) level was significantly higher in surrounding normal liver tissue[16]. It is general acknowledged that Zn has potent anti-oxidative ability, and Zn deficiency leads to enhanced oxidative stress and increased production of inflammatory cytokines. The oxidative stress may trigger harmful inflammatory response, and responsible for cancer[17]. The subjects we included in the present study have undergone chronic HBV infection before the onset of HCC, and HBV is capable of integrating into the host genome during the course of infection, and consequently lead to oncogene activation, tumor-suppressor gene inactivation, or other genome instability[18]. Interestingly, the HBV integration can be found in both HCC tissue and normal tissue[19]. However, the next-generation sequencing revealed that the viral integration occurred more frequently in HCC tissue than in paired non-tumor tissues[20]. We assume that the elevated integration level and chronic inflammation are able to disrupt the zinc/cooper ratio in liver tissue, and consequently produce free radical formation and further induce the damage of hepatocyte. Unlike Cu and Zn, cadmium (Cd) has been considered as a hazardous element to human and classified as a human carcinogen[21]. This toxic element is widely distributed in the human organs after absorption, with the major portion of the body burden located in the liver and kidney. By employing rat liver epithelial cell line, previous study observed the Cd-induced malignant transformation and significantly down-regulated the expression of apolipoprotein E (ApoE) which was recently established as a suppressor of cell invasion[22]. The source of exposure to Cd can be various, one of the most common one is smoking due to the high concentrations of Cd in tobacco plant, and despite the efforts of tobacco control, half of Chinese male were smokers according to the latest survey. Moreover, due to the absence of environmental regulation in early stage of Chinese industry, certain parts of soil were contaminated with the waste water, and the rice which is the staple food of Chinese population would accumulate high concentration of Cd. The pathway analysis also collaborates our hypothesis, as can be seen, the most enriched pathway in differentially expressed mRNA was mineral absorption (hsa04978). Based on the evidences above mentioned, we can conclude that the intake of some specific inorganic elements was involved with the development of HCC in Chinese male population. However, the further investigations such as epidemiological study focusing on inorganic elements exposure should be conducted to verify our assumption.

KIF20A, a member of Kinesin family, plays a critical role in cytokinesis[23], and more importantly, has been associated with the development and progression of various kinds of human cancers. The in vitro experiments

conducted by Gasnereau et al.[24] showed that the product of KIF20A mRNA MKlp2 was highly elevated in human hepatoma cell lines, while it can not be detected in normal human hepatocytes. The essential function of KIF20A involves with the cell adhesion, spreading, migration and proliferation. Other individual studies employing RNA-silencing method yielded conclusive results, and the cell viability and invasion capacity were significantly suppressed in cancer cell lines[25–26]. The findings of previous studies broadly supported the microarray analysis results of present study which demonstrated a 26.44-fold overexpression of KIF20A in HCC tissues when compared with normal tissues. On the other hand, the key molecular for the inhibition of migration and cell growth, HEPACAM was found to be 49.56-fold underexpressed in HCC tissues. The treatment of HEPACAM-overexpressing adenovirus in cancer cell line was able to promote the inhibitory effects on cell proliferation, viability and protein expression[27]. Based on the evidence obtained from in vitro experiment, the underlying mechanism of inhibitory effects caused by HEPACAM was involved with the inhibition of Wnt/β-catenin signaling pathway which was considered as cancer-related pathway if aberrantly activated[28]. The results reflected highly activation of cell migration and proliferation in malignant liver hepatocyte.

In the microarray analysis, we identified a large number of dysregulated lncRNAs which have not yet been reported in similar studies. With the subsequent qPCR validation conducted both in microarray analysis samples and in twice amount of independent samples, we observed high consistency between two methods. Among those differentially expressed lncRNAs, TCONS_00008984 exhibited astonishing elevation in HCC tissue with a 768.94-fold expression. Notably, this extreme high expression was not caused by few outliers, instead, it can be attributed to unanimous increase in qPCR validation. Given the extreme high elevation and absence of outlier, TCONS_00008984 has potential to serve as a diagnostic marker or prognosis indicator, despite its biological function remains clear currently. Epidemiological study focusing on the association between HCC clinical features and the expression level of TCONS_00008984 should be conducted to assess its capability of serving as a biomarker. In vitro experiment essential to reveal its underlying mechanism in the carcinogenesis of HCC should be performed as well.

In summary, the present study detected a great many differentially expressed lncRNAs and mRNAs by comparing HCC tissues and normal tissues with genome-wide microarray analysis, and subsequent qPCR validation showed high consistency. GO enrichment and pathway analysis revealed that the mRNAs related to inorganic ions response were involved with carcinogenesis, especially those associated with Zn and Cd. These findings suggested that the exposure to some specific inorganic elements may possibly involve with the risk of developing HCC, and such exposure should not be neglected. Epidemiological study should be implemented to investigate the synergic effect between inorganic elements exposure and chronic HBV infection, and subsequent interventions should be enhanced to reduce the incidence of HCC.

Declarations

Conflicts of interest
None to declare.
Competing interests
The authors declare that they have no competing interests to declare.
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Authors' contributions
ZXF and PGL collected the samples and analyzed the data. YL and JJN wrote the manuscript. The author(s) read and approved the final manuscript.
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Figures

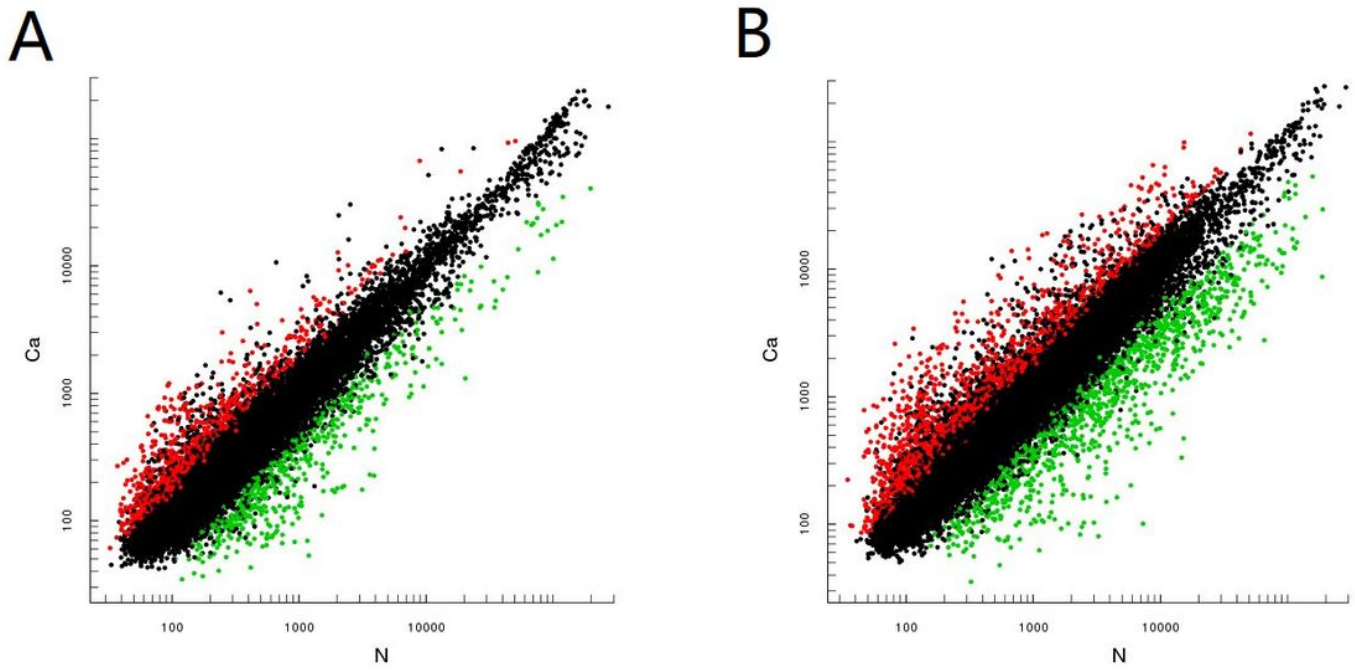

Figure 1

The scatter plot of (A) lncRNA and (B) mRNA expression signals in HCC and normal tissues

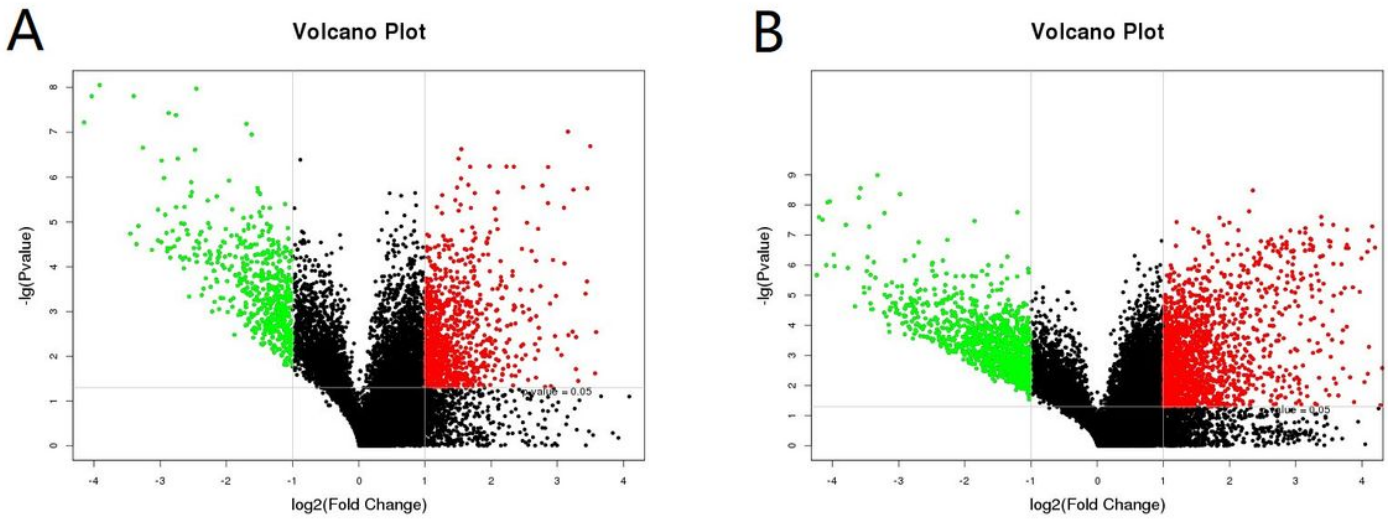

Figure 2

The volcano plot of (A) lncRNA and (B) mRNA expression signals in HCC and normal tissues

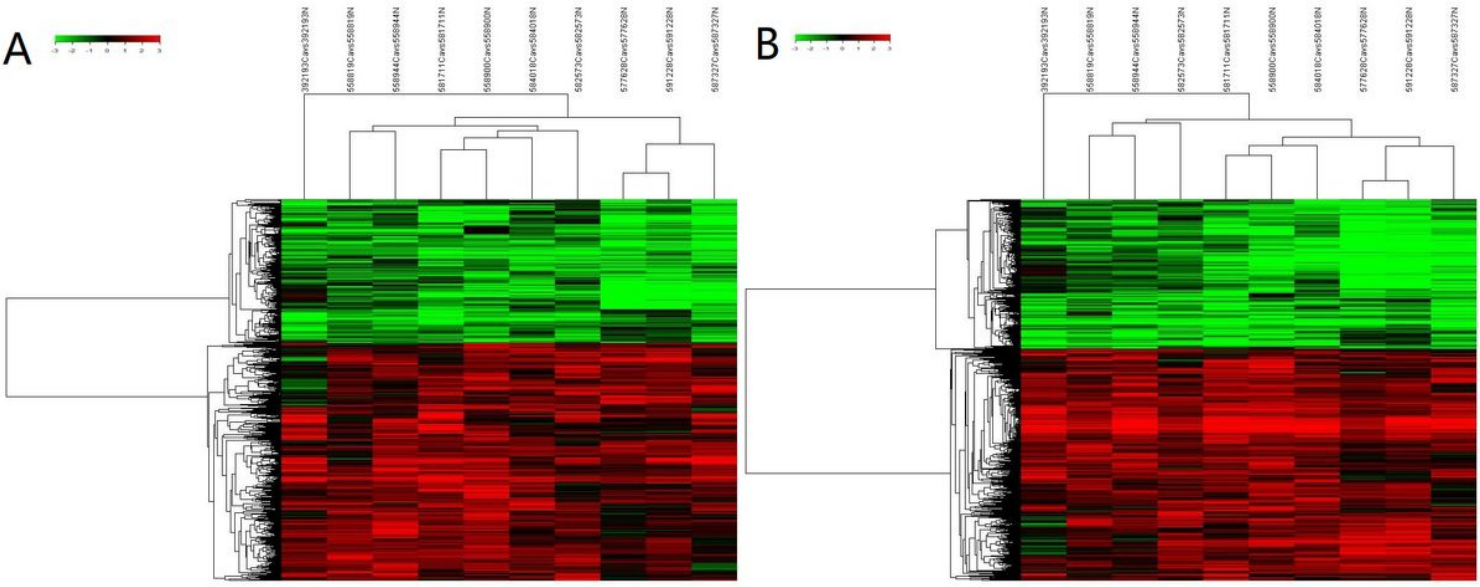

Figure 3

Hierarchical clustering on differential expression of (A) lncRNA and (B) mRNA in HCC and normal tissues

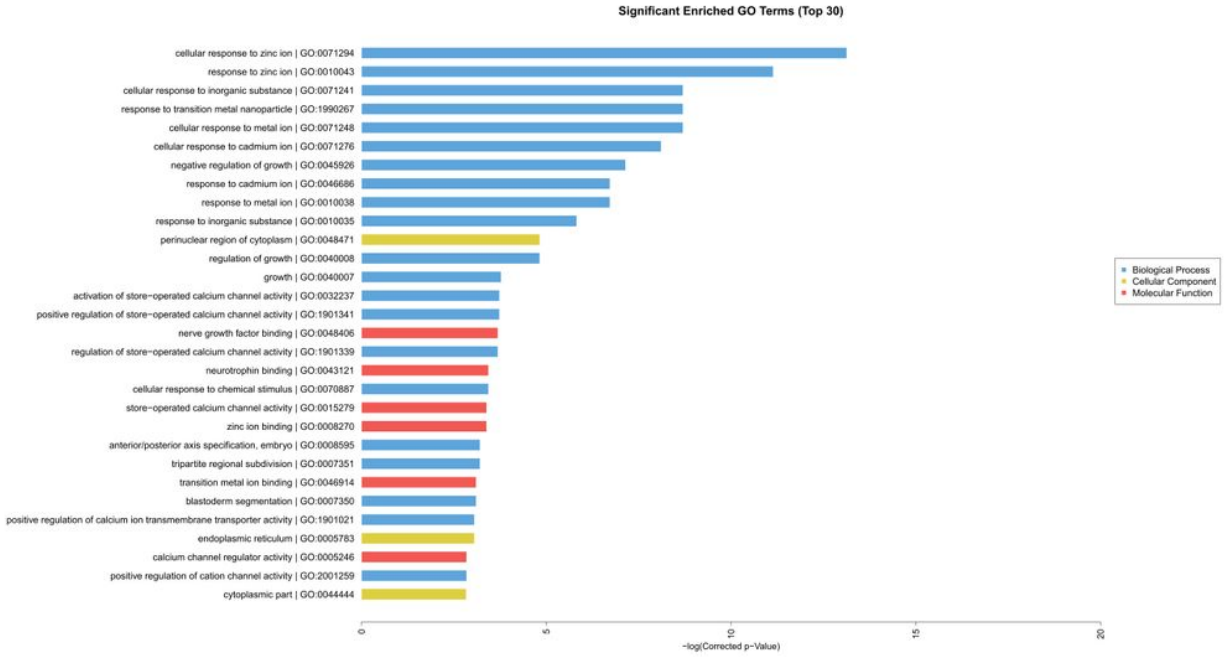

Figure 4

GO enrichment analysis of differentially expressed mRNAs

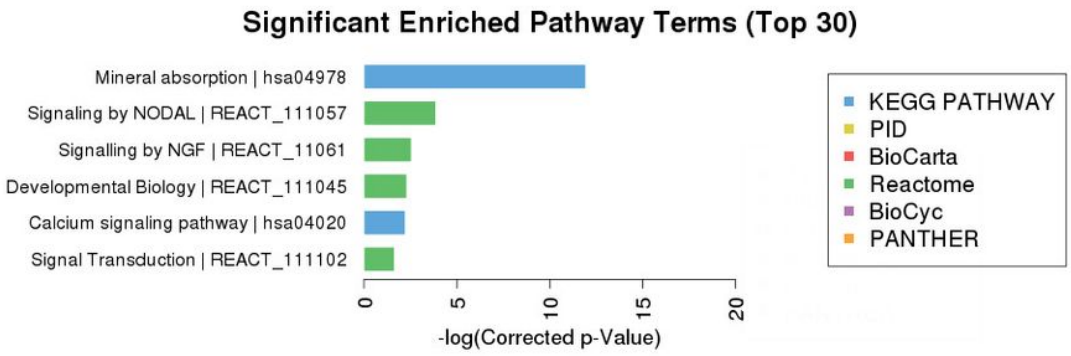

Figure 5

Pathway analysis of differentially expressed mRNAs

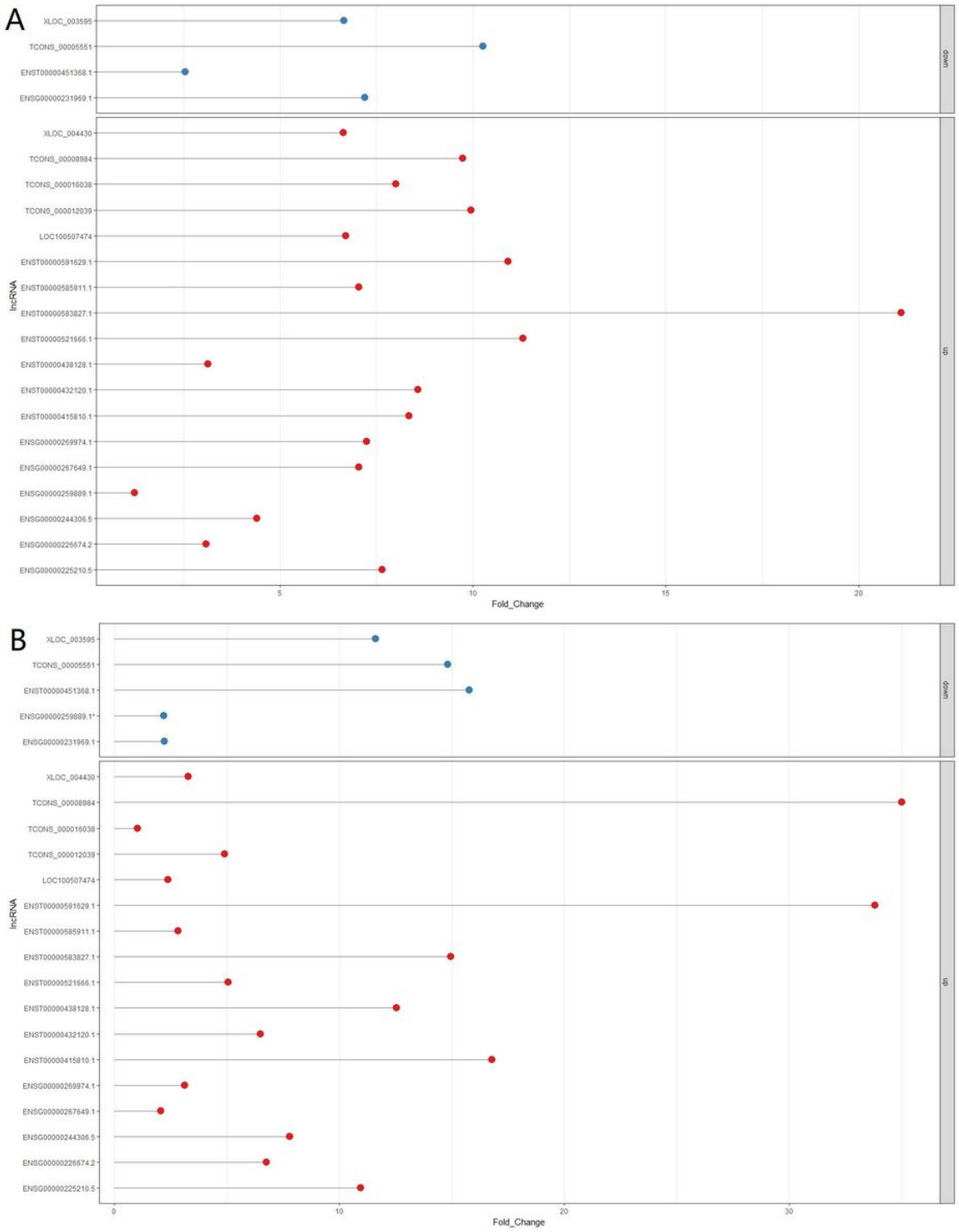

Figure 6

Fold change of 22 selected lncRNAs in (A) microarray analysis and (B) qPCR validation