LncRNAs expression signatures of hepatocellular carcinoma revealed by microarray

Wu-Tao Liu, Xin Lu, Guang-Hua Tang, Jin-Jun Ren, Wen-Jun Liao, Peng-Lei Ge, Jie-Fu Huang

Wu-Tao Liu, Xin Lu, Guang-Hua Tang, Jin-Jun Ren, Wen-Jun Liao, Peng-Lei Ge, Jie-Fu Huang, Department of Liver Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China

Author contributions: Liu WT contributed to the conception and design of the study, and wrote the manuscript; Tang GH, Ren JJ, Liao WJ and Ge PL performed the research; Huang JF contributed to the conception and design of the study, and approved the final version to be published; Lu X collected the conception and designed the study, revised the article and approved the final version to be published.

Supported by Grants from the China Medical Board of New York, No. 11-045; and National Key Technology Research and Development Program of China, No. BA106B01
Correspondence to: Xin Lu, MD, Professor of Surgery, Department of Liver Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No.1 Shuaifuyuan, Beijing 100730, China. luxinh@gmail.com
Telephone: +86-10-69156042 Fax: +86-10-69156043
Received: March 7, 2014 Revised: April 25, 2014
Accepted: May 12, 2014
Published online: May 28, 2014

Abstract

AIM: To analyze the expression profiles of long non-coding RNAs (lncRNAs) in hepatocellular carcinoma.

METHODS: Hepatocellular carcinoma (HCC) tissues and matched adjacent non-tumor (NT) liver tissues were collected from 29 patients with HCC, immediately after liver resection, between March 2011 and July 2013. The diagnosis of HCC was made based on histological examination. Differentially expressed lncRNAs between HCC and NT tissues were revealed through microarray-based lncRNAs expression profiling. Further, quantification of selected lncRNAs was performed using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR).

RESULTS: Six hundred and fifty-nine lncRNAs were differentially expressed between HCC and NT tissues, of which five [TCONS_00018278, AK093543, D16366, ENST00000501583, NR_002819 (MALAT1)] were selected for validation. Four of them were significantly downregulated in HCC tissues compared with NT tissues (P = 0.012, 0.045, 0.000 and 0.000, respectively), and the expression level of MALAT1 showed no significant difference (P = 0.114).

CONCLUSION: This study identified a set of lncRNAs differentially expressed in HCC tissues and provided useful information for exploring potential therapeutic targets and diagnostic biomarkers of this cancer.
© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Long non-coding RNA; Hepatocellular carcinoma; MALAT1; Expression signature; Microarray

Core tip: Long non-coding RNAs (lncRNAs) possess prominent and diverse regulatory functions in cancer processes. In this study, the expression profiles of lncRNAs in hepatocellular carcinoma (HCC) were analyzed using a microarray-based method. One hundred and seventy-one lncRNAs were significantly downregulated and 488 were upregulated in HCC tissues. Validation was performed in 29 subjects with HCC using quantitative real-time polymerase chain reaction and the expressions of four lncRNAs were significantly downregulated in HCC, whereas the expression of lncRNA MALAT1 showed no significant difference. The same result was obtained in a subset of patients with hepatitis B virus-related HCC.

Liu WT, Lu X, Tang GH, Ren JJ, Liao WJ, Ge PL, Huang JF. LncRNAs expression signatures of hepatocellular carcinoma revealed by microarray. World J Gastroenterol 2014; 20(20): 6314-6321 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i20/6314.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i20.6314
INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most prevalent neoplasm and the third leading cause of cancer-related death \(^{[4-6]}\). In particular, new cases of HCC in China account for almost 55% of all patients worldwide \(^{[4]}\). In the United States, the incidence of HCC is increasing faster than that of any other cancer \(^{[5]}\). HCC is strongly associated with chronic hepatitis B virus (HBV) and 50% of HCC cases are attributable to chronic infection with HBV \(^{[5,6]}\). With a dismal 5-year survival rate of approximately 5%-6%, HCC has one the worst prognoses among cancers and causes enormous social cost and burden, despite noteworthy advances in surgical techniques and medical treatment \(^{[5,6]}\). There is a great need to determine the relationships between clinicopathological features and molecular changes in HCC to develop new diagnosis and treatment strategies, and to improve the prognosis of diagnosed patients \(^{[6,9]}\).

The human genome comprises not only protein-coding genes, but also encodes a far larger set of non-protein-coding transcripts that have crucial functions for diseases \(^{[1,3,6]}\). Though much attention has focused on small non-coding RNAs [18-200 nucleotides (nt)] in recent years, long non-coding RNAs (lncRNAs), a newly discovered class of non-coding RNAs greater than 200 nt in length, have been shown to possess prominent and diverse regulatory functions in a wide range of biological processes \(^{[12-15]}\). Studies indicate that lncRNAs are associated with various human diseases, including cancer \(^{[16-19]}\). Altered lncRNAs expression levels correlate with patient clinicopathological features and prognosis. For example, Gupta et al. \(^{[20]}\) found that the expression of lncRNA HOX antisense intergenic RNA (HOTAIR) was increased in primary breast tumors and metastases, and the HOTAIR expression level in primary tumors was a powerful predictor of eventual metastasis and death. More recently, Yang et al. \(^{[21]}\) found that the expression level of lncRNA high expression in HCC (lncRNA-HEIH) in HBV-related HCC could effectively predict recurrence and was an independent prognostic factor for survival. Furthermore, using loss-of-function and gain-of-function approaches, they identified that lncRNA-HEIH plays a key role in cell-cycle regulation. In spite of this progress, studies on the roles of lncRNAs in HCC are still at a preliminary stage and their clinical significance remains largely unknown.

In this study, the lncRNA expression signatures from four subjects with HCC, were compared with matched adjacent non-tumor (NT) liver tissues. Five of these lncRNAs were evaluated by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) in twenty-nine HCC tissues and NT tissues. Our results demonstrated that lncRNAs profiles may provide new molecular biomarkers or therapeutic targets for HCC.

MATERIALS AND METHODS

Patients

In this prospective study, twenty-nine patients aged 30-73 years were enrolled from March 2011 to July 2013. The human ethics committees of the Peking Union Medical College Hospital (PUMCH) approved this study. Before surgery, written informed consent was obtained from all patients. All these patients received liver resection at PUMCH and were diagnosed as HCC histopathologically after surgery. There was no radiotherapy or chemotherapy before surgery. Four of these patients were used for microarray analysis of lncRNAs and twenty-five were used for an extra evaluation. Demographic and clinical characterizations of the study population are summarized in Table 1.

Tissue collection and total RNA extraction

Paired HCC tissues and adjacent NT liver tissues from every subject were snap-frozen in liquid nitrogen immediately after resection and stored at -80 °C until use. The mirVana™ RNA Isolation Kit (Applied Biosystems, Foster City, CA, United States) was used to extract total RNA from frozen samples, according to the manufacturer’s protocols, which was then eluted with 100 mL of nuclease-free water. A NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, United States) determined the RNA yield, and agarose gel electrophoresis with ethidium bromide staining evaluated the RNA integrity.

Microarray and computational analysis

Four HCC tissues and corresponding non-tumor liver tissues were used for lncRNA microarray profiling. 200 ng of total RNA was labeled using the LowInput Quick-Amp Labeling Kit, One-Color (24+) (Agilent, p/n 5190-2305, Santa Clara, CA, United States) and hybridized on the Gene Expression Hybridization Kit (Agilent, p/n 5188-4242). The Agilent human lncRNA array contains 46506 human lncRNAs probes and 30656 human mRNAs probes. Hybridization signals were detected using The Microarray Scanner (Agilent, p/n G2505C) detected the hybridization signals and the Feature Extraction Software (Agilent) analyzed the scanned images. OE Biotech, Shanghai, China performed the microarray work.

Real-time quantitative reverse transcription-PCR

A two-step reaction process was used for quantification: reverse transcription (RT) and PCR. Each RT reaction consisted of 0.5 µg RNA, 2 µL of PrimerScript Buffer, 0.5 µL of oligo dT, 0.5 µL of random 6 mers and 0.5 µL of PrimerScript RT Enzyme Mix I (TaKaRa, Japan), in a total volume of 10 µL. Reactions were performed in a GeneAmp® PCR System 9700 (Applied Biosystems) for 15 min at 37 °C, followed by heat inactivation of RT for 5 s at 85 °C. The 10 µL RT reaction mix was then diluted × 10 in nuclelease-free water and held at -20 °C.

Real-time PCR was performed using LightCycler®
Table 1  Demographic and clinical characteristics of patients with hepatocellular carcinoma for the study of tissue lncRNAs expression n (%)

| Parameters | Description | Minimum | Maximum |
|------------|-------------|---------|---------|
| Age (yr)   | 56.59 ± 10.97 | 30      | 73      |
| Gender     |             |         |         |
| Male       | 26          | (89.70) |         |
| Female     | 3           | (10.30) |         |
| Hepatitis virus infection |         |         |         |
| HBV        | 22          | (75.90) |         |
| HCV        | 3           | (10.30) |         |
| Negative   | 4           | (13.80) |         |
| Tumor size (cm) | 6.44 ± 3.65 | 2       | 19      |
| Tumor differentiation |         |         |         |
| Well       | 6           | (20.70) |         |
| Moderate   | 16          | (55.20) |         |
| Poor       | 7           | (24.10) |         |
| TNM stage  |             |         |         |
| I          | 10          | (34.50) |         |
| II         | 11          | (37.90) |         |
| III        | 7           | (14.0)  |         |
| N          | 1           | (4.0)   |         |
| BCLC stage |             |         |         |
| A          | 21          | (72.40) |         |
| B          | 1           | (0.40)  |         |
| C          | 7           | (24.10) |         |
| AFP (ng/mL) |             |         |         |
| < 400      | 17          | (58.60) |         |
| > 400      | 12          | (41.40) |         |
| PVTT       |             |         |         |
| Present    | 23          | (79.30) |         |
| Absent     | 6           | (20.70) |         |
| Tumor number |             |         |         |
| Single     | 27          | (93.10) |         |
| Multiple   | 2           | (6.90)  |         |
| Capsule invasive |         |         |         |
| Present    | 6           | (20.70) |         |
| Absent     | 23          | (79.30) |         |
| ALP (U/L)  | 42.90 ± 32.24 | 11   | 159     |
| ALB (g/L)  | 39.86 ± 3.72 | 28     | 47      |
| TBIL (µmol/L) | 15.36 ± 7.71 | 7.1   | 42.6    |
| DBIL (µmol/L) | 6.10 ± 3.08 | 3   | 16.5    |

Data are expressed as mean ± SD or n (%). TNM: Tumor-node-metastasis staging system; BCLC: The Barcelona Clinic Liver Cancer staging system; HBV: Hepatitis B virus; HCV: Hepatitis C virus; AFP: Alpha fetoprotein; ALP: Alkaline phosphatase; ALB: Albumin; TBIL: Total bilirubin; DBIL: Direct bilirubin.

RESULTS

LncRNAs expression profiles in HCC

LncRNA profiling detected 659 lncRNAs with significant differential expression levels in HCC tissues compared with paired NT tissues (> 2-fold change; P < 0.05): 488 were significantly upregulated and 171 were significantly downregulated in HCC tissues. The list of the top twenty differentially expressed lncRNAs identified by microarray analysis is shown in Table 3. Unsupervised hierarchical clustering analysis showed systematic variations in the expression of the 659 lncRNAs and 690 mRNAs between paired HCC and NT tissues. The samples were self-segregated into the HCC and NT clusters. These normalized microarray expression data were used to generate heat maps (Figure 1).

Overview of the mRNA profile in HCC

Through 30656 coding transcripts probes, up to 13664 mRNAs could be detected in four pairs of tissue samples. The expression levels of 577 mRNAs were significantly upregulated in HCC tissues compared with the matched NT tissues, while the expression levels of 113 mRNAs were significantly downregulated (> 2-fold change; P < 0.05) among all four pairs of samples. Gene ontology (GO) and pathway analysis showed that the differentially expressed mRNAs might be involved in regulation of transcription, and cell cycle and cell division-associated signal pathways. These results support the view that carcinogenesis of HCC is a complex multistep process involving many altered signaling cascades that lead to a heterogeneous molecular profile.

Validation of profiling data using quantitative real-time polymerase chain reaction

To validate the microarray analysis results, five lncRNAs were randomly selected from the differential lncRNAs and their expressions were analyzed using qRT-PCR in 29 pairs of HCC and matched NT tissues (Figure 2A). Our data indicated that the expressions of TCONS_00018278, AK093543, D16366 and ENST00000501583 were significantly downregulated in HCC (P = 0.012, 0.045, 0.000 and 0.000, respectively), whereas the expression of NR_002819 showed no sig-


**Table 2** The primer sequences used in this study

| Target ID       | Forward primer                  | Reverse primer                  |
|-----------------|---------------------------------|---------------------------------|
| TCONS_00018278  | ATGAGGAACAGCGTCTTTTG          | GATTGAATGAGGGTCCAT              |
| AK093543        | GCAGATTGTAACACCTAGGCA         | ACCTTCTACAGGGTTGATTG           |
| D16366          | TGCAAATTCGGGGTCCGA            | AGAGAACCCACAGTACGCC            |
| ENST0000501583  | TCACACTCTACACCTGGCA          | TCGTCTACGCTGGGTT              |
| NR_002819       | CAGCAGACGACAGGGTTC           | TCCITCCACAAATCGGCAC           |
| GAPDH           | TGGTCCCATCAATGCCCTTT         | CTACACGCTACTTCACTGCGG        |

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

**Table 3** The top twenty differentially expressed lncRNAs identified by the microarray in four hepatocellular carcinoma patients

| lncRNAs       | Fold change (Ca/Nt) | Regulation direction |
|---------------|--------------------|----------------------|
| BC015396      | 23.924885          | Up                   |
| NR_046337     | 15.497891          | Up                   |
| ENST0000542490 | 11.481871 | Up                   |
| Hs.568152     | 10.981759          | Up                   |
| ENST0000548057 | 10.762795 | Up                   |
| D16366        | 10.744071          | Down                 |
| ENST0000548036 | 10.218715 | Up                   |
| uc001888-3    | 10.091635          | Up                   |
| TCONS_00000610 | 9.758178 | Up                   |
| TCONS_00000616 | 8.632748 | Up                   |
| AK021443      | 7.991496           | Up                   |
| AY027770      | 7.947163           | Up                   |
| TCONS_00007547 | 7.920387 | Up                   |
| Hs.171192     | 7.900335           | Up                   |
| ENST0000568976 | 7.852221 | Up                   |
| TCONS_00000613 | 7.546310 | Up                   |
| XR_110225     | 7.462819           | Down                 |
| ENST0000500112 | 6.609479 | Up                   |
| ENST0000548475 | 6.573556 | Up                   |
| ENST0000527297 | 6.532015 | Up                   |

Hepatocellular carcinoma samples (Ca) vs matched non-tumor (Nt).

LncRNAs, which are non-coding RNAs greater than 200 nt in length, were first described by Brockdorff et al.[28]. The authors described a 19 kb non-protein-coding transcript, X-inactive specific transcript (XIST), which is responsible for the inactivation of one of the two X chromosome in placental females through DNA methylation. Since then, especially in recent years, an increasing number of studies have reported that lncRNAs are involved in the progression of diverse diseases, especially cancers.[22, 23, 24]. A great number of lncRNAs can be commonly detected in various tumors, such as colon cancer-associated transcript (CCAT1), metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), HOTAIR and H19.[25, 26, 27]. Accumulating evidence suggests that unique lncRNAs are associated with the development and progression of cancer through diverse pathways, including regulation of the cell cycle,[28], apoptosis,[29], metastasis,[30], autophagy.[31] and epigenetic regulation[32] in tumor tissues or malignant cell lines. Thus, lncRNAs have opened a new field of cancer genomics. Although there are no drugs that act against lncRNAs presently, it will be fascinating to observe whether drugs could be developed that specifically target lncRNAs. Notably, lncRNAs can be detected in human body fluids and hold great promise as biomarkers.[30, 32, 33].

In this study, we focused on exploration and description of the microarray-based lncRNAs expression signatures of HCC. With abundant and varied probes amounted to 46506 lncRNAs and 30656 mRNAs in the microarray, a large number of lncRNAs could be determined quantitatively. Comprehensive in-depth analysis of the expression profiles of lncRNAs in HCC was executed to understand the role of lncRNAs in HCC. On the basis of the lncRNA array data, we confirmed that tens of thousands of lncRNAs were expressed; however, only a few hundred of them were differentially expressed in each tissue sample. In all four HCC tissues, 171 lncRNAs downregulated and 488 were upregulated compared with adjacent non-tumor tissues. Most of these differentially expressed lncRNAs have not been characterized functionally. They might be involved in the regulation of the development and progression of HCC and might be helpful in exploring the molecular basis of HCC.

To validate the microarray expression findings, five lncRNAs (TCONS_00018278, AK093543, D16366, ENST0000501583, NR_002819) were subjected to qRT-PCR...
PCR in twenty-nine pairs of samples. The results showed significantly lower expression of TCONS_00018278, AK093543, D16366 and ENST00000501583 in HCC tissues compared with matched NT tissues. So far, little is known about the roles of these four lncRNAs in diseases. However, based on their significantly downregulated expression levels in HCC tissues, we assumed that some of them may be associated with the pathogenesis or progression of HCC, which should be explored in future studies.

Our data indicated that the expression of NR_002819 showed no significant differences between tumor tissues and adjacent non-tumor tissues either in all twenty-nine HCC patients ($P = 0.114$) or in the subgroup of twenty-two HBV-related HCC patients ($P = 0.141$).

NR_002819, generally known as MALAT1 or nuclear-enriched abundant transcript 2, is a highly abundant nucleus-restricted RNA localized to nuclear speckles, which influences alternative splicing of pre-mRNAs by modulating the cellular distribution and activity of SR splicing factors.$^{23,39,44}$ Overexpression of MALAT1 was first found in non-small cell lung cancers and then in many other cancer types, such as breast, pancreas, colon, prostate, implying key roles in carcinogenesis and tumor metastasis.$^{23,26,41}$
Currently, little is known about the function and mechanism of MALAT1 in HCC. Luo JH and his colleagues found that the expression level of MALAT1 was significantly higher in hepatoblastoma (HBPL) tissues than HCC tissues. Lin et al. found that MALAT1 expression in human carcinomas vs the corresponding non-tumor tissues was markedly elevated in four patients with HCC. Lai et al. found that MALAT1 was upregulated in HCC tissues compared with adjacent non-tumor tissues in sixty patients, which correlated with HCC prognosis. Liu et al. failed to find a significant association between single nucleotide polymorphisms (SNPs) in MALAT1 and decreased susceptibility to HCC in HBV persistent carriers in a case-control study involving over 3000 subjects. In vitro studies have demonstrated that MALAT-1 interacts with hnRNP C and regulates the progress of the cell cycle in HepG2 and HeLa cell lines.

The present study, and the studies are the only three papers focusing on the quantitative assessment of MALAT1 in HCC tissues. The discrepancy between our findings and those of Lin et al. and Lai et al. suggested that further validation is necessary. It should be noted that the sample sizes of all of these three studies are comparatively small. For instance, project of Lai et al. included 60 patients and only four subjects were involved in study. Follow-up studies in larger cohorts of patients should be conducted at a later date.

The results of qRT-PCR were consistent with the data from the microarray and revealed that there were unique lncRNAs expression signatures in these tissues. These differentially expressed lncRNAs represent a potential novel way to detect HCC. Some studies revealed the existence of lncRNAs in body fluids, such as serum and plasma. However, it is too early to utilize this collection of lncRNAs as biomarkers of HCC based on the present data. Further studies to investigate their functions and structures are necessary. The data obtained from this present research will benefit the exploration of novel diagnostic markers and therapeutic targets in HCC. The sample size of our pilot study was small and only five lncRNAs were selected for validation, so further studies in larger cohorts of patients are necessary.

This study described the lncRNAs expression signatures of HCC based on a microarray, which provided a promising new opportunity to detect HCC. Also, this study identified a four dysregulated lncRNAs in HCC. Our data indicated that the expression of MALAT1 showed no significant differences in HCC tissues. These results warrant further analysis of the relationship between lncRNAs and clinicopathological features of HCC, which will help our understanding of its carcinogenic mechanisms, and permit us to explore new early diagnostic markers and novel target genes to prevent HCC development.

**REFERENCES**

1. Bruix J, Llovet JM. Prognostic prediction and treatment strategy in hepatocellular carcinoma. *Hepatology* 2002; 35: 519-524 [PMID: 11870363 DOI: 10.1053/jhep.2002.32089]
2. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; 379: 1245-1255 [PMID: 22535262 DOI: 10.1016/s0140-6736(11)6347-0]
3. Nguyen VT, Law MG, Gore GJ. Hepatitis B-related hepatocellular carcinoma: epidemiological characteristics and disease burden. *J Viral Hepat* 2009; 16: 453-463 [PMID: 19302335 DOI: 10.1111/j.1365-2893.2009.01117.x]
4. Shariff MI, Cox IJ, Gomaa AI, Khan SA, Gedroyc W, Taylor- Robinson SD. Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis and therapeutics. *Expert Rev Gastroenterol Hepatol* 2009; 3: 353-367 [PMID: 19673623 DOI: 10.1586/erg.09.35]
5. Hao K, Luk JM, Lee NP, Mao M, Zhang C, Ferguson MD, Lamb J, Dai H, Ng IO, Sham PC, Poon RT. Predicting prognosis in hepatocellular carcinoma after curative surgery with common clinicopathologic parameters. *BMC Cancer* 2009; 9: 389 [PMID: 19886989 DOI: 10.1186/1471-2407-9-389]
6. But DY, Lai CL, Yuen MF. Natural history of hepatitis-related hepatocellular carcinoma. *World J Gastroenterol* 2008; 14: 1652-1656 [PMID: 18350595 DOI: 10.3748/wjg.14.1652]
7. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* 2011; 61: 212-236 [PMID: 21685461 DOI: 10.3322/caac.20121]
8. Willyard C. Researchers look for ‘sweet’ method to diagnose cancer. *Nat Med* 2007; 13: 1267 [PMID: 17987006 DOI: 10.1038/nm1107-1267]
9. Imbeaud S, Ladeiro Y, Zucman-Rossi J. Identification of
novel onco-genes and tumor suppressors in hepatocellular carcinoma. *Semin Liver Dis* 2010; 30: 75-86 [PMID: 20175035 DOI: 10.1055/s-0030-1247134]

10 Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functions, fates and sequences from the RNA world. *Genes Dev* 2009; 23: 1494-1504 [PMID: 19571179 DOI: 10.1101/gad.180909]

11 Zhou H, Xiao B, Zhou F, Deng H, Zhang X, Lou Y, Gong Z, Du C, Guo J. MIR-421 is a functional marker of circulating tumor cells in gastric cancer patients. *Biomarkers* 2012; 17: 104-110 [PMID: 22263628 DOI: 10.3109/1354750X.2011.614961]

12 Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; 136: 215-233 [PMID: 19167326 DOI: 10.1016/j.cell.2009.01.002]

13 Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. *Cell* 2009; 136: 642-655 [PMID: 19293886 DOI: 10.1016/j.cell.2009.01.035]

14 Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* 2010; 11: 597-610 [PMID: 20661255 DOI: 10.1038/nrg2843]

15 Yang F, Bi J, Xue X, Zheng L, Zhi K, Hua J, Fang G. Up-regulated long non-coding RNA H19 contributes to proliferation of gastric cancer cells. *FEBS J* 2012; 279: 3159-3165 [PMID: 22776265 DOI: 10.1111/j.1742-4658.2012.08694.x]

16 Tahira AC, Kubsrus M, Faria MF, Dazzani B, Fonseca RS, Maracaja-Coutinho V, Verjovski-Almeida S, Machado MC, Reis EM. Long noncoding intronic RNAs are differentially expressed in metastatic and metastatic pancreatic cancer. *Cancer* 2011; 10: 141 [PMID: 20728386 DOI: 10.1186/1476-4598-10-141]

17 Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell* 2007; 129: 1311-1323 [PMID: 17604720 DOI: 10.1016/j.cell.2007.05.022]

18 Qureshi IA, Mattick JS, Mehler MF. Long non-coding RNAs in nervous system function and disease. *Brain Res Rev* 2010; 1338: 20-35 [PMID: 20380817 DOI: 10.1016/j.brainres.2010.03.110]

19 Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell* 2009; 136: 629-641 [PMID: 19293885 DOI: 10.1016/j.cell.2009.02.006]

20 Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin domains in human HOX loci by noncoding RNAs. *Cell* 2009; 136: 1083-1096 [PMID: 20395366 DOI: 10.1038/nn8975]

21 Yang F, Zhang L, Luo XS, Yuan JH, Xu D, Yuan SX, Zhu N, Zhou H, Wang J, Huang J, Sun Y. Long non-coding RNA metastasis associated in lung adenocarcinoma transcript 1 derived miniRNA as a novel plasma-based biomarker for diagnosing prostate cancer. *Eur J Cancer* 2013; 49: 2949-2959 [PMID: 23726266 DOI: 10.1016/j.ejca.2013.04.026]

22 Yoo EJ, Zhang L, Zhi K, Gu Y, Fang G. Long noncoding RNA CCAT1, which could be activated by c-Myc, promotes the progression of gastric carcinoma. *J Cancer Res Clin Oncol* 2013; 139: 437-445 [PMID: 23143645 DOI: 10.1007/s00432-012-1324-x]

23 Khahtain D, Dinger ME, Mazar J, Crawford J, Smith MA, Mattick JS, Perera RJ. The melanoma-upregulated long non coding RNA SPRY4-IT1 modulates apoptosis and invasiveness. *Cancer Res* 2011; 71: 3852-3862 [PMID: 21588391 DOI: 10.1158/0008-5472.CAN-10-4460]

24 Yang F, Huan XS, Yuan SX, Zhang L, Zhou WP, Wang F, Sun SH. Repression of the long non coding RNA-LET by histone deacetylase 3 contributes to hypoxia-mediated metastasis. *Mol Cell* 2013; 49: 1083-1096 [PMID: 23390020 DOI: 10.1016/j.molcel.2013.01.010]

25 Ying L, Huang Y, Chen H, Wang Y, Xia L, Chen Y, Liu Y, Qiu F. Downregulated MEG3 activates autophagy and increases cell proliferation in bladder cancer. *Mol Biotechnol* 2013; 9: 407-411 [PMID: 23529831 DOI: 10.1007/s12033-012-9328-5]

26 Yang Y, Li H, Hou S, Hu B, Liu J, Wang J. The noncoding RNA expression profile and the effect of Non-coding RNA AK126668 on cisplatin resistant in non-small-cell lung cancer cell. *PLoS One* 2013; 8: e65309 [PMID: 23741487 DOI: 10.1371/journal.pone.0065309]

27 Augoff K, McCabe B, Plow EF, Sossey-Alaoui K. miR-31 and its host gene IncRNA LOC554202 are regulated by promoter hypermethylation in triple-negative breast cancer. *Mol Cancer* 2012; 11: 5 [PMID: 2288555 DOI: 10.1186/1476-4598-11-5]

28 Gaughwin PM, Ciesla M, Lahiri N, Tribraz S, Brundin P, Björkqvist M. Hsa-mir-34b is a plasma-stable microRNA that is elevated in pre-manifest Huntington’s disease. *Hum Mol Genet* 2011; 20: 2225-2237 [PMID: 21421997 DOI: 10.1093/hmg/ddr111]

29 Xie H, Ma H, Zhou D. Plasma HULC as a promising novel biomarker for the detection of hepatocellular carcinoma. *Biomol Res Int* 2013; 2013: 136106 [PMID: 23762823 DOI: 10.1155/2013/136106]

30 Hutchinson JN, Esmenring AW, Clemson CM, Lynch CR, Lawrence JB, Chess A. A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. *BMc Genomics* 2007; 8: 39 [PMID: 17270048 DOI: 10.1186/1471-2164-8-39]

31 Anko ML, Müller-McNicoll M, Brandt H, Curk T, Gorup C, Henry I, Ule J, Neugebauer KM. The RNA-binding landscapes of two SR proteins reveal unique functions and binding to diverse RNA classes. *Genome Biol* 2012; 13: R17 [PMID: 22818241 DOI: 10.1186/1471-2105-13-5]
41 Tano K, Akimitsu N. Long non-coding RNAs in cancer progression. *Front Genet* 2012; 3: 219 [PMID: 23109937 DOI: 10.3389/fgene.2012.00219]

42 Luo JH, Ren B, Keryanov S, Tseng GC, Rao UN, Monga SP, Strom S, Demetris AJ, Nalesnik M, Yu YP, Ranganathan S, Michalopoulos GK. Transcriptomic and genomic analysis of human hepatocellular carcinomas and hepatoblastomas. *Hepatology* 2006; 44: 1012-1024 [PMID: 17006932 DOI: 10.1002/hep.21328]

43 Lin R, Maeda S, Liu C, Karin M, Edgington TS. A large non-coding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. *Oncogene* 2007; 26: 851-858 [PMID: 16878148 DOI: 10.1038/sj.onc.1209846]

44 Lai MC, Yang Z, Zhou L, Zhu QQ, Xie HY, Zhang F, Wu LM, Chen LM, Zheng SS. Long non-coding RNA MALAT-1 overexpression predicts tumor recurrence of hepatocellular carcinoma after liver transplantation. *Med Oncol* 2012; 29: 1810-1816 [PMID: 21678027 DOI: 10.1007/s12032-011-0004-z]

45 Liu Y, Pan S, Liu L, Zhai X, Liu J, Wen J, Zhang Y, Chen J, Shen H, Hu Z. A genetic variant in long non-coding RNA HULC contributes to risk of HBV-related hepatocellular carcinoma in a Chinese population. *PLoS One* 2012; 7: e35145 [PMID: 22493738 DOI: 10.1371/journal.pone.0035145]

46 Yang F, Yi F, Han X, Du Q, Liang Z. MALAT-1 interacts with hnRNP C in cell cycle regulation. *FEBS Lett* 2013; 587: 3175-3181 [PMID: 23973620 DOI: 10.1016/j.febslet.2013.07.048]

47 Reis EM, Verjovski-Almeida S. Perspectives of Long Non-Coding RNAs in Cancer Diagnostics. *Front Genet* 2012; 3: 32 [PMID: 2248643 DOI: 10.3389/fgene.2012.00032]
