Microarray analysis of microRNA expression in liver cancer tissues and normal control

Lianjie Lin *, Yan Lin, Yu Jin, Changqing Zheng

Department of Gastroenterology and Hepatology, Shengjing Hospital of China Medical University, 36 Sanhao, Heping, Shenyang 110004, China

A R T I C L E   I N F O

Article history:
Accepted 20 February 2013
Available online 10 April 2013

Keywords:
Liver cancer
miRNA analysis
hsa-miR-421

A B S T R A C T

Background: Recently, many studies have focused on microRNAs (miRNAs) expression profiling in liver cancer, due to the ability of these small RNAs to potently influence cellular behavior. In this study, to further investigate the relationship between them, the miRNA expression profiling of the cancer liver tissues and normal liver tissues were compared.

Methods: The datasets of miRNAs microarray in liver cancer and normal control were downloaded from Gene Expression Omnibus. Then the SOAP analysis was performed to identify the differentially expressed miRNAs.

Results: A total of 221 differentially expressed miRNAs were found. Five of them (including hsa-miR-15b, hsa-miR-1975, hsa-miR-199a-3p, hsa-miR-199b-3p and hsa-miR-421) were determined by t-test and may be involved in the pathogenesis of liver cancer.

Conclusion: There differently expressed miRNAs may be potential molecular markers for liver cancer screening.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Liver cancer is one of the most frequent cancer diseases which grow on the surface or inside the normal liver. Liver cancer accounts for the third most common cause of cancer-related deaths worldwide, especially in parts of Asia and Africa with estimated >680,000 new cases and half million of deaths annually (Yi et al., 2008). Further, the liver cancer processes are controlled by a complex regulatory network (Eifert and Powers, 2012). Therefore, a better understanding of mechanism of liver cancer development is urgently needed.

Genome-wide approaches have reshaped the landscape of cancer research. Meta-analysis of multi-study data has allowed the identification of differentially expressed genes that may have biomarker potential (Chan et al., 2008). Emphasis on genome-wide gene expression analyses has been driven by the general view that alterations in protein-coding oncogenes or tumor-suppressor genes underlie tumor genesis (Sarver et al., 2009). However, the discovery of a growing class of small non-coding RNAs, termed miRNAs, has opened a new field of cancer research and revealed the complexity of cancer biology (Sarver et al., 2009). MiRNAs are short, non-coding RNAs, about 21 nucleotides in length, that can bind to the 3′-untranslated region (3′-UTR) of target transcripts and regulate gene expression by degradation of the target mRNA or inhibition of its translation (Lee et al., 1993). Recently, many studies have focused on mRNA expression profiling in liver cancer. Aberrant expression of miRNAs in liver often leads to cancer development, such as miR-125b and miR-122 (Hou et al., 2011; Huang et al., 2011). It is reported that miR-125b can suppress liver cancer cell growth, migration and invasion (Liang et al., 2010). On the other hand, loss of miR-122 expression in liver cancer often correlates with suppression of the hepatic phenotype and gain of metastatic properties (Coulouarn et al., 2009; Wen and Friedman, 2012). Nonetheless, researches of relationship between the miRNAs and liver cancer development using high throughput method remain rare (Lee et al., 2002; Chan et al., 2008).

Short Oligonucleotide Alignment Program (SOAP) is one of the first methods published for the mapping of short tags, in which both tags and genome are first of all converted to numbers using 2-bits-per-base encoding. SOAP is compatible with numerous applications, including single-read or pair-end resequencing, small RNA discovery and miRNA tag sequence mapping (Li et al., 2008). In this study, SOAP analysis was performed to identify the differentially expressed miRNAs in the liver cancer tissue compared to normal control. A total of 221 differentially expressed miRNAs were found, which suggest that these miRNAs may play an important role in liver cancer development. Anyway our work will improve our understanding of the mode of action of miRNAs in liver cancer.

2. Materials and methods

2.1. Data selection and processing

Microarray datasets of miRNA in liver cancer tissues and normal control were accessible at National Center for Biotechnology Information...
Gene Expression Omnibus (NCBI GEO, http://www.ncbi.nlm.nih.gov/geo/) database under the accession numbers: GSM531975, GSM531976 (human normal liver tissue sample), GSM531977 (HBV-infected liver tissue), GSM531982, GSM531984, GSM531986 (HCV (+) hepatocellular carcinoma tissue sample), and GSM531988 (HBV (−)/HCV (−) hepatocellular carcinoma tissue sample) (Basyuk et al., 2003).

2.2.2 Mapping the sequencing data to genome and miRNA

SOAP (Li et al., 2008) was used to align raw reads to human genome (hg19) and human miRNA data base (miRBase; release 13.0). The sum of the read counts and the TPM (transcript per million) were calculated.

2.3. Differentially expressed miRNAs

Student's t-test was performed to identify differentially expressed miRNAs between cancer tissues and normal tissues. To avoid inducing false positive results, only the P values < 0.01 were considered as differentially expressed miRNAs.

3. Results

3.1. Mapping the sequencing data to genome and miRNA

SOAP was performed to align raw sequencing reads to human genome (hg19) and the miRNA database (miRBase: release 13.0). We counted the read mapping to the miRNAs location and calculated the TPM (transcript per million). Finally, a total of 703 differentially expressed miRNAs were obtained. The general view of sample differences were analyzed by principle component analysis (PCA) for normal liver tissue samples and liver cancer samples (Fig. 1). As shown in Fig. 1, the HBV (−)/HCV (−) hepatocellular carcinoma tissue was far away from the normal liver tissue or HBV–HCV infected hepatocellular carcinoma tissue. We should also like to conclude that the virus infected hepatocellular carcinoma tissue is much different from the virus negative hepatocellular carcinoma tissues and the results may imply that we should take different therapeutic methods for virus infected liver cancer. Those miRNAs with a minimum average TPM of 10 were chosen as highly expressed miRNAs (the threshold of P values < 0.01). Finally, using the proposed cut-off criteria, 221 highly expressed miRNAs were found.

3.2. Differentially expressed miRNAs

Log transformation was firstly applied to transform the TPM expression to log expression for all miRNAs across samples. Then t-test was performed to detect the differential expression miRNAs. At last, five significantly differential expression miRNAs were obtained including hsa-miR-15b, hsa-miR-1975, hsa-miR-199a-3p, hsa-miR-199b-3p and hsa-miR-421. The expressed levels of these miRNAs are shown in Fig. 2. Among these five differentially expressed miRNAs, hsa-miR-1975, hsa-miR-421 and hsa-miR-15b were up-regulated in cancer liver tissues, while hsa-miR-199a-3p and hsa-miR-199b-3p were down-regulated in liver cancer tissues. They potentially play important roles in liver cancer development.

4. Discussion

MicroRNA is a kind of post-transcriptional regulator that binds to complementary sequences of target miRNAs, mainly resulting in translational repression or target degradation and gene silencing (Bartel, 2009; Kusenda et al., 2009). Mis-regulation of miRNA expression may contribute to human disease such as infection and cancer (Calin et al., 2002; He et al., 2005). Therefore, studying the liver cancer related miRNAs by high throughput method will provide the ground work for the further study of the molecular mechanism of liver cancer.

Here, total 7 miRNA expression profiles from the original dataset were selected and then the SOAP analysis was performed to align raw reads to human genome (hg19) and human miRNA database (miRBase). Moreover, five of them (hsa-miR-15b, hsa-miR-1975, hasmiR-199a-3p, hsa-miR-199b-3p and hsa-miR-421) were determined by t-test, of which hsa-miR-1975, hsa-miR-421 and hsa-miR-15b were up-regulated, and hsa-miR-199a-3p and hsa-miR-199b-3p were down-regulated in liver cancer tissue. These miRNAs may be involved in cancer pathogenesis.

The hsa-miR-421 is confirmed to be a key regulator in gastric cancer. In 73.33% of the gastric cancer samples, miR-421 is over-expressed. Inhibition of miR-421 expression decreases the growth of both MGC-803 and SGC-7901 gastric cancer cells in vitro (Jiang et al., 2010). The hsa-miR-421 is also reported to regulate DNA damage-induced cell cycle S-phase checkpoints. The miR-421 overexpression overcomes the IR (irradiation) induced DNA synthesis block and mimics the radio-resistant DNA synthesis of A-T cells. The miR421-induced continuous DNA synthesis is also seen with lower doses of IR at 2 and 5 Gy. MiR-421 overexpression does not alter the proliferation rate of HeLa cells but increase post-IR cell death. Furthermore, the effect of miR-421 on the cell cycle S phase checkpoint and radio-sensitivity is proven to be mediated through Ataxia-telangiectasia mutated (ATM) (Hao et al., 2011). In liver cancer, miR-421 is demonstrated to target 3’ untranslated region of human farnesoid X receptor mRNA to downregulate its expression, promoting the proliferation, migration, and invasion of hepatocellular carcinoma cells (Zhang et al., 2012).

The hsa-miR-15b can regulate cell cycle progression by targeting cyclins in glioma cells. Over-expression of mir-15b results in cell cycle arrest at G0/G1 phase while suppression of mir-15b expression causes a decrease of cell populations in G0/G1 and a corresponding increase of cell populations in S phase (Xia et al., 2009). Defects in a cell cycle checkpoint often lead to cancer process (Hartwell, 1992).

The hsa-miR-199a-3p is involved in ovarian cancer process by regulation of IKKβ. IKKβ is a major factor promoting the TLR-MyD88-NF-κB pathway that confers to ovarian cancer cell the capacity to constitutively secrete proinflammatory cytokines and therefore promoting tumor progression and chemoresistance (Chen et al., 2008). Restoring attenuated levels of miR-199a-3p in liver cancer cells leads to G1-phase cell cycle arrest, reduced invasive capability, enhanced susceptibility to hypoxia, and increased sensitivity to doxorubicin-induced apoptosis (Fornari et al., 2010). Further study report that mir-199a-3p may reduce proliferation of hepatocellular carcinoma cell lines by targeting CD44 (Henry et al., 2010). Our findings are consistent with these results, demonstrating miR-199a-3p may be an important regulator in liver cancer process.
Although hsa-miR-1975 (Youssef et al., 2011; Yu et al., 2012) and hsa-miR-199b-3p have been identified in cancer tissues (Hiroki et al., 2010) (including liver cancer in this study), the detail molecular mechanism remains unclear. Therefore, further experiments are needed to be performed.

5. Conclusions

In this study, a total of 221 differentially expressed miRNAs were identified between the liver cancer tissues and normal control. Five of them were determined by t-test, which are much more likely to be potential regulators in liver cancer process. Anyway our findings correspond with the former studies, and will be a valuable resource to the liver cancer research community for further studies. However, further experiments are still needed to confirm the biological function of these miRNAs.

Conflict of interest

None.

Acknowledgments

This work was supported partly by the Science and Technology Program of Liaoning Province (2010225008); the Ph.D. Start-up Foundation of Liaoning Province (20081048); and the Science and Technology Program of Shenyang (F10-205-1-17).

References