

Stability analysis on liver cancer related miRNA in Serum

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MicroRNAs (miRNA) are non-coding, single-stranded RNAs of ~22 nucleotides and constitute a novel class of gene regulators that are found in both plants and animals. Recent evidence has shown that miRNA mutations or aberrantly expression correlate with various human cancers and indicated that miRNAs can function as tumour suppressors and oncogenes. While many studies have focused on miRNA expression in physiological and pathological processes, variables related to miRNA for new serum biomarkers have simultaneously emerged. Now miRNA has been applied to early detection of cancer and monitoring of cancer recovery by using detection of peripheral blood.

Up to present, there are no reports regarding of liver cancer specific miRNA biomarkers in serum for the great threat of liver cancer to human life. Therefore a systemic research on the characteristic of miRNA is quite necessary.

Liver cancer Huh-7 cell-line, liver tumor tissues and clinical serum samples were performed in the role of experiment material. A systemic treatment, such as different temperature(-80°C, -20°C, 4°C, room temperature and 37°C) treated for 3h, in room temperature treated for 0, 1, 3, 6, 12, 24 hours, RNase A treated for 0, 3, 6, 12 hours incubation in 37°C, DNase I treated for 0, 3, 6, 12 hours incubation in 37°C, different free-thaw cycles (0, 2, 5, 7, 10 cycles) treated, different pH value (control, pH=1, 6, 9, 13) of solution treated for 3h incubation in 37°C were performed before Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) analysis with 40 cycles. Furthermore, liver cancer related miRNAs were detected in each reaction. We used 18S rRNA as control gene. All the results indicated that 18S rRNA was fragile for decreased relative expression sharply. MiRNAs could be resistant to harsh conditions simultaneously. The p-value indicated the repeatability of the data by using the Student's t-test. $P < 0.05$ was considered to be significant.

Meanwhile, we evaluated the Pearson's correlation coefficient of liver cancer related miRNAs expression of 22 healthy human subjects by qRT-PCR. Expression levels of serum miRNAs were reproducible and consistent among 22 healthy human subjects for the R value was access to 1, p-value ≤ 0.05 . The result was considered to be significant. Pearson correlation scatter plot of the relative serum miRNAs expression between male and female of R^2 was 0.0953. Results suggested that miRNA expression is not correlated between genders.

Taken together, these results implied that liver cancer related miRNA (miRNA-21, -25, -29c, -93, -198, -221, -222) expression levels in serum were quite stable, also present and detectable, reproducibly consistent among individuals of the same species in serum. They will be potential for serum liver cancer biomarkers in future.

On the other side, it is difficult to obtain abounding and high-quality of RNA in serum. Our tests have shown that pre-heating procedure is a robust serum RNA extraction method and efficient for RNA isolation. This method is also essential for further serum source microRNA study. In fact, after qRT-PCR, the C_T (threshold cycle) value decreased at least 5. In conclusion, based on our study, pre-heating provided an optimized protocol for serum RNA isolation.

Biography

Xianfeng Ding is an associate professor of Biology, and is Director of the Biological Science Experiment center at the Zhejiang Sci-Tech University. Xianfeng Ding holds a BS degree in chemistry from Shanxi Normal University and a MD degree in Chemistry from Jiangnan University; Pursuing research in the interdisciplinary areas of chemistry and biology, she did some research biochip technology for novel methods in emerging life and medical science fields by biochip technology at Dr. Gao' lab at University of Houston, seeking for a potential molecular biomarker for liver injury, correlation and quantitation of microRNA aberrant expression in tissues and sera from different tumors, research has been continuously funded by the National High-Tech Research & Development Program of China (Grant No. 2007AA02Z165) and Zhejiang Nature Science Foundation of China (Grant No. Y2100681). She has published more than 20 papers in reputed journals, has more than six issued and pending patents and is general manager of biotechnology companies with success in product commercialization.