MicroRNA differential expression profile in cholangiocarcinoma cell line and normal bile duct cell line

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Abstract: The aim of this study was to explore microRNA differential expression profile between cholangiocarcinoma cell line QBC939 and normal bile duct cell line, for further studies on functions of microRNA in pathogenesis of the cholangiocarcinoma. Six samples from QBC939 cell line and another 6 samples from normal bile duct cell line were chosen as the experimental group and the control group, respectively. MiroRNA profiles in these samples were analyzed by microarray. The threshold value used to screen up and down regulated microRNA were Fold Chang>2. Six microRNAs were found up-regulated in the experimental group were more than 8 times compared with control group. In addition, 5 microRNAs were found down-regulated 8 times compared with control group. These differential expressions of miroRNA might be related to the formation and metastasis of cholangiocarcinoma.

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1. Introduction

MicroRNA is a kind of conservative non-coding RNA,with a length of 19 to 25 nucleotide. It is widely distributeed in virus, plant and higher mammal. The research shows that microRNA plays the key role in controlling development, apoptosis, multiplication and differentiation of the cells. Due to the close relations between its unbalance expression and the tumor, the relations between microRNA and malignant tumor become a hot spot[1-3]. Cholangiocarcinoma is a kind of malignant tumor derived from bile duct epithelium in or out of the liver. In recent years, along with the development of imaging diagnostic technology, the detection rate of cholangiocarcinoma increases. It is reported that in the Primary Tumors of Liver, cholangiocarcinoma ranks only second to liver cancer. As the formation and development of cholangiocarcinoma is a complex process with various steps and factors, the pathogenesis is still not so clear. The correct diagnosis in early stage and timely reasonable treatment has important significance to improve the long-term survival rate for the cholangiocarcinoma patient. However, its clinical manifestation is not so typical that it brings great difficulty to early diagnosis and treatment, and it is often the case that the condition has been in the late fall when patients are with obvious clinical symptoms. The proposing of microRNA provides a new concept to the pathogenesis, early diagnosis and targeted treatment [4, 5]. Applying microRNA chip technology to screen the differential expressed microRNAs from bile duct cancer cell strains QBC939 and normal bile duct epithelia cell strains, this research provides important theoretical foundation for further study in the forming mechanism and effective treatment means of cholangiocarcinoma.

2. Material and Methods

The materials are purchased from the following company: cholangiocarcinoma cell line QBC939 and normal bile duct epithelia cell line are all from CAMS(Chinese Academy of Medical Sciences), McCoy'5A medium, high glucose DMEM medium, and super and standard fetal calf serum are from American HyClone Company, Peniciline /Streptomycin liquor and trypsin are from GIBCO company, cell RNA total extraction reagent Trizol is from American Invitrogen company, MiRCURY ^{TM} chip is from Denmark Exiqon company, Axon GenePix 4000B hydrone RNA chip scanistor is from American Melecular Devices company, PCR thermal cycler is from Japanese Takara Bioengineering Co. Ltd, and RT-PCR primer is composed by Life Technologies.

Cholangiocarcinoma cell line QBC939 and normal bile duct epithelia cell line are cultivated in the 37℃ 5 % CO2 incubator with 10% fetal calf serum McCoy'5A medium and 10% super fetal calf serum medium, respectively. Six samples from QBC939 cell line and another 6 samples from normal bile duct cell line were chosen as the experimental group and the control group, respectively, and

numbered as Q-1、Q-2、Q-3、Q-4、Q-5、Q-6 and H-1、H-2、H-3、H-4、H-5、H-6。

Use Trizol to extract total RNA of the 12 cell sample which are in the logarithmic growth phase, then determine the absorbance value D(260) 、 D(280)and D(230) with ultraviolet spectrophotometer, and then quantify the total RNA while calculate OD260/280 value and OD260/230 value, combing formaldehyde degeneration gel electrophoresis to analyze the quality of RNA.

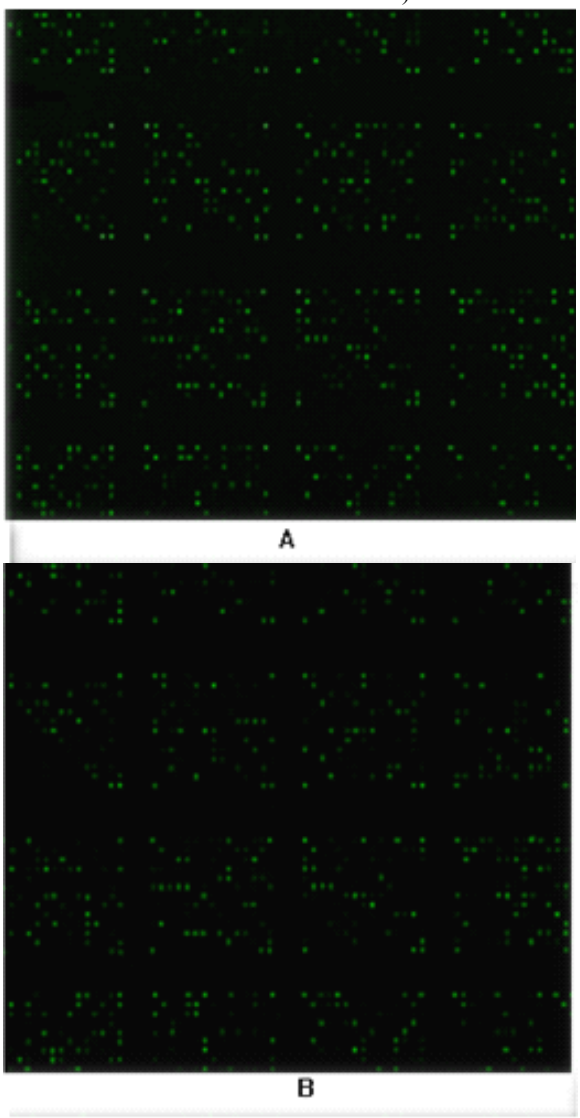
Take 5 μg of total RNA from the 12 samples to prepare fluorescently tagged probe, use miRCURY LNA™ to mark kit and fluorescently tagged microRNA using marker enzyme with Hy3™/Hy5™, obtaining the fluorescent light so as to hybridize the chip. Under standard conditions, using PhalanxTM thermal contraction hybridizing bag, hybridized the marked probe and miRCURY™ chip (with each sample detected 4 times by the chip). After hybridized, take out the chip and wash it, scanning it immediately while the chip is dry, and then the fluorescence intensity of the chip is scanned. Then mating software is applied to do the analysis of image and data, and the differential expressed gene ($P<0.05$) is screened out, and lastly, cluster analysis to these differential genes is done.

MicroRNA Chip data employs GenePix Pro6.0 software to design the t-test grouply according to fluorescence intensity after the extraction of the original data, and P<0.05 has statistic meaning. The PCR data adopts 2 - △△ CT to do the analysis and determines up-regulation, down-regulation and range of the molecular to be examined according to whether 2 - △△ CT is greater or less than zero.

3. Results

In order to detect whether the total RNA of the 12 samples can meet the following demand of the chip experiment, the OD260/280 value and OD260/230 value are determined by ultraviolet spectrophotometer and the total RNA quality is analyzed through observing the electrophoretogram. The results detected by ultraviolet spectrophotometer are as follows: the OD260/280 values of the total 12 samples of Q-1、2、3、4、5、6 and H-1、2、3、4、5、6 are 2.02、2.08、2.04、2.03、2.09、2.02、2.02、2.03、2.05、2.07、2.03、2.09, respectively, which are all between 1.8 and 2.1. The result shows that there is no degradation, protein and DNA pollution. The OD260/230 value of the 12 samples are 2.15、2.18、2.09、2.13、2.12、2.21、2.12、2.15、2.19、2.10、2.18、2.14, respectively, which are all above 2.0. The result shows that there is no pollution of organic solvent. The electrophoresis result shows that the total RNA of two groups of sample all have clear 18S and 28S strip, and the quality of the sample preservation are good. All of the results show that the total RNA quality fit the following microRNA demand of chip experiment.

The hybridized chip is scanned by GenePix 4000B (Picture 1). Compared to the normal bile duct epithelia cell line, the obviously differential expression of microRNA up-regulated or down-regulated two times of cholangiocarcinoma cell line totals 133, among them, 72 microRNA are above 2 times, 6 microRNA are above 8 times(Table 1); and 61 microRNA are below 2 times,5 microRNA are below 8 times with $P<0.05$（Table 2).



Picture 1 part of the chip scanning picture of sample microRNA (A represents the Q-1microRNA chip scanning picture of cholangiocarcinoma cell line, while B represents H-1microRNA chip scanning picture of normal cell line)

Table 1. MicroRNA with the ratio of the differential expression between cholangiocarcinoma cell line QBC939/ normal bile duct epithelia cell line above 8 times

microRNA with expression up-regulated 8 times		
Name	times	P
hsa-miR-21	91.7	*0.003*
hsa-miR-141	76.9	*0.002*
hsa-let-200b	48.5	*<0.001*
hsa-miR-15a	24.4	*0.017*
has-miR-23a	21.6	*<0.001*
hsa-miR-15b	10.8	*0.006*

Table 2. MicroRNA with the ratio of the differential expression between normal bile duct epithelia cell line / cholangiocarcinoma cell line QBC939 above 8 times

microRNA with expression down-regulated 8 times		
Name	times	P
hsa-miR-125a	89.6	*<0.001*
hsa-miR-31	85.2	*<0.001*
hsa-let-7b	49.1	*<0.001*
hsa-miR-95	24.8	*<0.001*
has-miR-320	17.9	*<0.001*

4. Discussions

MicroRNA is widely existed in various kinds of creatures. There are about 1,000 kinds of microRNAs in the human body at present; they regulate most protein-coded gene and a part of non-protein-coded gene. It is estimated that microRNA takes up about 3% human being's gene in the way of controlling the expression of mRNA by multiple microRNA corresponding to one mRNA[6, 7]. These microRNAs inhibit the expression of targeted gene through inducing the incision and degradation of mRNA, translated inhibition or other forms of regulatory mechanism[8]. It has been proven that microRNA plays tremendous regulatory role in the formation of various tumors[9-11]. According to its different roles, these microRNAs can be divided into carcinogenicity microRNAs and anti- carcinogenicity microRNAs. The cancer gene may be promoted by the up-regulated microRNAs in the tumor tissues, while inhibited by the down-regulated ones. Calin found that microRNA-15a and microRNA-16a have down-regulated or lost expression in most chronic lymphocytic leukemia patient. Soon afterwards microRNA was found disorderly expressed in lung cancer, thyroid caner, breast cancer, gastrointestinal cancer, liver cancer and so on successively. At present, these microRNAs have been classified into oncogene or anti-oncogene according to their role in the tumor. Through appling the microRNA chip to analyze the differential expression profiling of microRNA between cholangiocarcinoma cell line QBC939 and normal bile duct epithelia cell line, this research screens out 6 microRNA which have differential expression up-regulated 8 times in the cholangiocarcinoma cell lines, they are microRNA-200b , microRNA-21 , microRNA-141 , microRNA-15a , microRNA-23a and microRNA-15b respectively ; 5 microRNAs which have differential expression downregulated 8 times, and they are microRNA-125a , microRNA-31 , microRNA-95 , microRNA-320 and let-7b, respectively. The result shows that the imbalanced expression of these microRNAs has closely relationship with the growth, apoptosis and invasion of the cholangiocarcinoma cell lines.

The family of microRNA-200 includes the member of microRNA-200a , microRNA-200b , microRNA-200c , microRNA-141 , microRNA-429 and so on. The research has been shown that microRNA-200 family may enhance the expression of E- calcium-adhere protein through inhibit the expression of ZEB1 、 ZEB2 so as to play the important part in the process of transferring epithelial cell and mesenchyme. This research finds that in the cholangiocarcinoma cells, there are expressions of microRNA-200b and microRNA-141 in the microRNA-200 family. The target gene of microRNA-200b is PTPN12, which can inhibit the cancer through dephosphorylating to c-Ab1 and Src. The target gene of microRNA-141 is CLOCK, which not only plays important role in regulating the biorhythm of the organisms, but also inhibits cell division and promotes cell apoptosis as anti-oncogene, so we can consider that microRNA-141 promotes the proliferation of cholangiocarcinoma cells through inhibiting the expression of CLOCK. MicroRNA-21 has unusual expression in various malignant tumors[12], and most anti-oncogenes are the direct target genes of microRNA-21, such as TPM1 , PDCD4 and maspin. The inhibition to the expression of anti-oncogenes not only benefit the growth of the tumor cells, but also plays important role to the invasion and transference of tumor. This research finds that microRNA-21 has obviously high expression in cholangiocarcinoma cell lines, which shows that microRNA-21 and cholangiocarcinoma have close relations. In addition, microRNA-23a 、 microRNA-15b 、 microRNA-125a , microRNA-

31，microRNA-95，microRNA-320 and let-7b have obviously differential expression between cholangiocarcinoma cell lines and normal bile duct epithelia cell line, which hints that they also play important role in the formation and development of cholangiocarcinoma, and their role needs to be further studied.

Appling microRNA chip technology, this research sets up the expression chart of microRNA in cholangiocarcinoma, and the differentiate microRNA screened out may be used as the biology marker of screening, diagnosing and prognosis monitoring of cholangiocarcinoma. However, the present research is still in the initial stage so that new microRNAs related with cholangiocarcinoma needs to be further searched. At the meantime, the functions of all kinds of microRNAs also need to be further explored.

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