FXR expression in a rat model of hilar cholangiocarcinoma

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Research article

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Abstract

Background: To develop a rat model of hilar cholangiocarcinoma and detect Farnesyl X receptor (FXR) expression in hilar cholangiocarcinoma tissues of this model, in order to provide a new method for the treatment of hilar cholangiocarcinoma.

Methods: Forty male Wistar rats (body weight, 185 ± 5 g) were randomly divided into two groups (n = 20 each) as follows: The control group was fed a standard diet, and the experimental group was injected by cholangiocarcinoma QBC939 cell suspension along the hilar bile duct into the bile duct bifurcation with microsyringe. Every day note the rats' mental state, diet, and fur condition. At 4 weeks, one rat of the experimental group was sacrificed, and we recorded changes in hilar bile duct size, texture, and form. This procedure was repeated at 6 weeks. After 6 weeks, hilar cholangiocarcinoma developed only in the experimental group, thereby establishing an experimental model for studying QBC939-induced hilar cholangiocarcinoma. Tumor formation was confirmed by pathological examination, and hilar bile duct tissues were harvested from both the groups. A real-time polymerase chain reaction assay and an immunohistochemical assay were used to analyze the expression of *FXR* in hilar cholangiocarcinoma and normal hilar bile duct tissues.

Results: From the second week, the rats in experimental group began to eat less, and their body mass decreased compared with controls. After 6 weeks, we detected hilar cholangiocarcinoma in 17 rats (85%) in the experimental group. In the experimental group, we found that the levels of total cholesterol, total bilirubin, and direct bilirubin were higher compared with those of the control group. Simultaneously, muddy stones emerged from the bile ducts of rats in the experimental group. The *FXR/Gapdh* mRNA ratio in hilar cholangiocarcinoma and normal hilar bile duct tissues differed markedly. Light microscopy revealed a granular pattern of *FXR* expression which reacted with the anti-*FXR* antibody. Each section was randomly divided into six regions, with 80 cells were observed in every region. Sections with >10% positive cells were designated positive,Sections with <10% positive cells were designated negative. Each group included 4,800 cells. In the experimental group, 1,196 cells (24.9%) were positive and 3,538 cells (73.7%) were positive in the control group, and this difference was statistically significant.

Conclusion: *FXR* expression significantly decreased in hilar cholangiocarcinoma of rats than in those of controls, suggesting that drugs targeting *FXR* may be a new strategy for hilar cholangiocarcinoma.

Background

Hilar cholangiocarcinoma is a malignant tumor in the digestive system, and the patients who die from this disease increases every year. Unfortunately, treatment methods are limited. The preferred method is Surgery , but it has contraindications. Only part of patients are eligible for radical resection, and some patients must undergo palliative resection, however the 5-year survival rate is low. Numerous patients are not eligible for surgery because of factors such as tumor infiltration, or metastasis ,multiple organ

dysfunction . Alternative therapies include radiotherapy, chemotherapy, and radio frequency ablation; however, they do not improve survival rates. Therefore, new therapies are urgently required.

Gene therapy for cancer is not widely used, but it has great potential. Therefore, we searched for genes involved in the pathogenesis of hilar cholangiocarcinoma that may serve as therapeutic targets. Farnesyl X receptor (*FXR*) is related with bile acid metabolism, which is important in bile acid secretion. In former experiment we have detected that bile salt export pump (*Bsep*), a target gene of *FXR,*expression decreased in hilar cholangiocarcinoma tissues of rats, which led us to investigate the role of *FXR,* whether the role of *FXR* is enhanced or diminished in hilar cholangiocarcinoma[1-3].

Methods

Rats

It were provided by the Animal Test Center of Southwest Medical University.We randomly divided 40 Wistar rats (male, 185± 5g) into two equal groups that received a normal diet group (control group) and a injection by cholangiocarcinoma QBC939 cell suspension(experimental group).Before conducting the study, the rats were healthy and were not administered a diet containing drugs.

Experimental methods

Materials:DMEM culture medium(Sigma Inc.),Pentobarbital sodium(Shanghai Xinyu Biotechnology Co., Ltd.),QBC939 human cholangiocarcinoma cell line(Shanghai Yubo Biotechnology Co., Ltd.),Microsyringe(Shanghai Fuguang Precision instrument Co., Ltd.).Frozen tissue sections were prepared using a cryostat, HFsafe biological safety cabinet, microscopic imaging system (PM-10A), etc. The main reagents were real-time PCR kits, anti- *FXR* monoclonal antibody, etc.

The tumor cells were cultured in DMEM medium at 37℃and 5% saturated humidity.Selected cells with good growth to inoculate Wistar rats.The diameter of the needle tip of the microsyringe is 40μm,connected to the 1ml syringe through a rubber tube.

Establish animal model The cultured cholangiocarcinoma QBC939 cells were prepared to cell suspension with a concentration of 1×106 cells / ml. Wistar rats in the experiment group were anesthetized with 1.5% pentobarbital sodium and 0.2ml/100g intraabdominal injection.After disinfection, the abdomen was cut along the linea alba.After the tumor cell suspension was adsorbed by microsyringe,the needle of microsyringe punctured along the hilar bile duct into the bile duct bifurcation, and 100 μl of tumor cell suspension was injected.Pressed to stop the bleeding, closed the abdomen in turn, and end the operation.The control rats were fed a standard diet throughout the course of the study. Every day note the rats' mental state, diet, and coat condition. At 4 weeks, one rat of the experimental group was sacrificed after it was administered anesthesia(with 4% pentobarbital sodium and 150mg/1kg intraabdominal injection), and we recorded changes in hilar bile duct size, texture, and form. This procedure was repeated at 6 weeks. Tumor formation was confirmed according to the findings of

pathological examination, hilar cholangiocarcinoma tissues and hilar bile duct tissues were harvested from the groups for the analysis of *FXR* mRNA expression using real-time PCR(RNA was extracted from cancer and normal hilar bile duct tissues using Trizol reagent. An ABI7500 real-time PCR detection system was used, and Gapdh served as the internal control. real-time PCR kits was used(SR1100)) and *FXR* protein expression using immunohistochemistry(anti-FXR antibody was used(CHEMICON)).

Statistical analysis

SPSS22.0 statistical software was used for data analysis. Data are presented as the mean ± SD. The t test was used to judge the differences between two groups, and P <0.05 indicates statistically significance differences. The $\chi 2$ test was used to evaluate immunohistochemistry data, and P<0.05 indicates statistically significance differences.

Results

From the second week, the rats in the experimental group began to eat less, and their weights decreased compared with controls (Tables 1, 2). Two rats in the experimental group died after 6 weeks. There were no fatalities in the control group. After 6 weeks, through pathologic examination we detected hilar cholangiocarcinoma in the hilar bile duct of 17 rats (85%) in the experimental group (Figure 1). The analyses of bile samples of the groups are shown in Table 3.

Analysis of FXR expression

The sense and antisense primers used to detect *FXR* mRNA were as follows: 5'-CCTCATTGTCTCCCCGACTTA-3' and 3'-GCCTCTAGAAAGCAGTGTTCA-5'. The sense and antisense primers used to detect *Gapdh* mRNA were as follows: 5'- GATGGTGGGTATGGGTCAGAA-3' and 3'-CTAGGAGCCAGGGCAGTAATC-5'.The $2^{-\Delta\Delta Ct}$ method was used to express the data. In cancerous and normal bile duct tissues the *FXR*/ *Gapdh* ratios were 16 and 35, respectively, after eight cycles, and the difference between groups was statistically significant (t = 2.392, $P < 0.05$) (Figure 2).

Light microscopy revealed a granular pattern of *FXR* protein expression which reacted with the anti-*FXR* antibody(Figure 3). Each section was randomly divided into six regions, with 80 cells were observed in every region. Sections with >10% positive cells were designated positive, Sections with <10% positive cells were designated negative. Each group included 4,800 cells. In the experimental group, 1,196 cells (24.9%) were positive and 3,538 cells (73.7%) were positive in the control group, and this difference was statistically significant ($\chi 2 = 10.35$, $P < 0.05$).

Discussion

In digestive system the malignant tumors include pancreatic cancer, gastric cancer, primary liver cancer and others. Hilar cholangiocarcinoma exhibit one of the most malignant phenotype. Despite during several years the diagnostic and therapeutic modalities developed in clinical and basic research, the

pathogenesis of hilar cholangiocarcinoma, the changes in the pathological and physiological characteristics of tumors, and the changes of the tumor environment are not completely clear. Factors that are associated with the pathogenesis of hilar cholangiocarcinoma include Calculus of bile duct, congenital cholangiectasis, Chinese liver fluke and primary sclerosing chola. However, the mechanisms that regulate specific signal transduction pathways associated with the malignant phenotype of hilar cholangiocarcinoma are not clear. For example, hilar cholangiocarcinoma may be caused by specific factors that accumulate and interact each other, therefore which can explain the lack of effective treatments. Usually, if the tumor is small or limited to part of the hilar bile duct ,it can be completely resected, patients have a favorable prognosis. However, if the tumor infiltrates surrounding tissues or metastasizes, surgery is impossible. The effects of interventions such as chemotherapy, radiofrequency ablation, radiotherapy, and interventional embolization are limited. Moreover, the rates of recurrence and metastasis are high, and the 5-year survival rate is low. Therefore, more effective diagnostic and therapeutic modalities are urgently required. Although gene therapy is not widely used, it has good prospect. So, we searched for genes involved in the pathogenesis of hilar cholangiocarcinoma that may serve as therapeutic targets[4-6].

FXR usually express on the surface of liver cells and bile duct cells, and its function is to transport bile acid. Therefore, *FXR* plays an important role in bile-acid excretion, bile-acid concentration stabilization, and the reabsorption process of bile acid enterohepatic circulation[7-10]. We previously found that bile salt export pump (*Bsep*), a target gene of *FXR*,expression decreased in hilar cholangiocarcinoma tissues of rats. Therefore, we tried to analyze if the expression levels of *FXR*, changed similarly in hilar bile duct tissues of rats with hilar cholangiocarcinoma[11-13].

The course of bile acid enterohepatic circulation requires various transporters to interact each other. At first bile acid synthesis by hepatocytes, with the regulation of *FXR* bile acid is exported to the intestinal tract by the bile salt export pump[14,15]. After bile acid is discharged into the small intestine, approximately 95% of the conjugated bile acid is reabsorbed through the apical sodium-dependent bile acid transporter, ileum bile acid binding protein, and terminal apical sodium-dependent bile acid transporter. sodium/ taurocholate cotransporting polypeptide(*Ntcp*) mediates approximately 80% of bile acids into liver cells[16,17], which are again secreted into the bile to formate enterohepatic circulation of bile acids[18-20].

If the levels of *FXR* expression are inappropriate, bile acid secretion and reabsorption disorders may occur, and bile will accumulate in the bile duct[21-23]. So cholesterol and bile pigments may accumulate in bile ducts, leading to the formation of stones. The long-term presence of a calculus in the bile duct is one of possible causes of promoting the growth of hilar cholangiocarcinoma. Therefore, it is important to gain a better understanding of *FXR* expression in hilar cholangiocarcinoma tissues[24-26].

In the present study, we established a rat model of hilar cholangiocarcinoma. After 6 weeks, hilar cholangiocarcinoma developed only in the experimental group, thereby establishing an experimental model for studying QBC939-induced hilar cholangiocarcinoma.The dietary intake and weights of rats in

the experimental group were lower than those of the control group.The frequency of rats with hilar cholangiocarcinoma was 85%. In the experimental group with hilar cholangiocarcinoma, the levels of total cholesterol, total bilirubin, and direct bilirubin were higher than those of the control group. Simultaneously, muddy stones emerged from the bile ducts of rats in experimental group, and the levels of expression of *FXR* were lower in the rats with hilar cholangiocarcinoma than in those in the control group.Thus, we speculate that if increased quantities of bile acids in bile ducts, the expression of *FXR* will increase[27,28], which will accelerate the secretion of bile acid to maintain its concentrations. However, in hilar cholangiocarcinoma bile acid secretion is greatly reduced, So bile deposits in the bile duct and potential stone formation. that induces inflammation of the bile duct. Repeated destruction and proliferation of bile duct cells increase the probability of an oncogenic events and the emergence of cells with the malignant phenotype.

There are several problems with the drugs used to treat hilar cholangiocarcinoma.

For example, their use is limited because they do not kill all the tumor cells, require large doses, adversely affect the digestive system, and are poorly tolerated. Drugs in the research and development stages do not directly target the genes that contribute to hilar cholangiocarcinoma. The data presented here may enhance our understanding of the molecular basis of hilar cholangiocarcinoma[29,30]. Moreover, our study illuminates that *FXR* maybe a target for new and more effective treatment strategies.

Conclusions

In summary, our study reveals that FXR espression increase in a rat model of hilar cholangiocarcinoma.Which illuminates that FXR maybe a target for new and more effective treatment strategies.

List Of Abbreviations

FXR:Farnesyl X receptor, Bsep: bile salt export pump, Ntcp:sodium/ taurocholate cotransporting polypeptide

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the affiliated hospital,Southwest Medical University,Luzhou, Sichuan Province, China.All methods were carried out in accordance with animal experiment guidelines and regulations.

Consent to publish

All authors have approved the manuscript and agree with publication in Journal of BMC Cancer.

Availability of data and material

The data and material have availability to the manuscript

Competing interests

The authors have no competing interests to declare.

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Not applicable

Authors' contributions

ZMY and WJP designed and coordinated the research; HK performed the majority of experiments and analyzed the data; ZMY and XXM wrote the paper.All authors have read and approved the manuscript.

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Tables

Due to technical limitations the Tables are available as a download in the Supplementary Files.

Figures

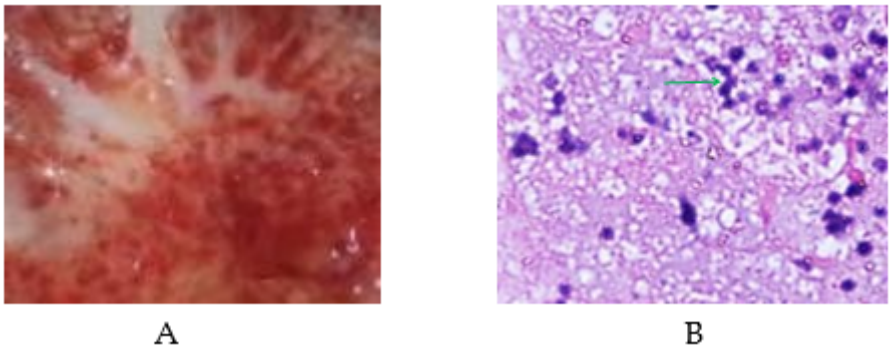

Figure 1

Pathological examination. A) hilar cholangiocarcinoma. B) A tumor cell is indicated by the green arrow (100× magnification).

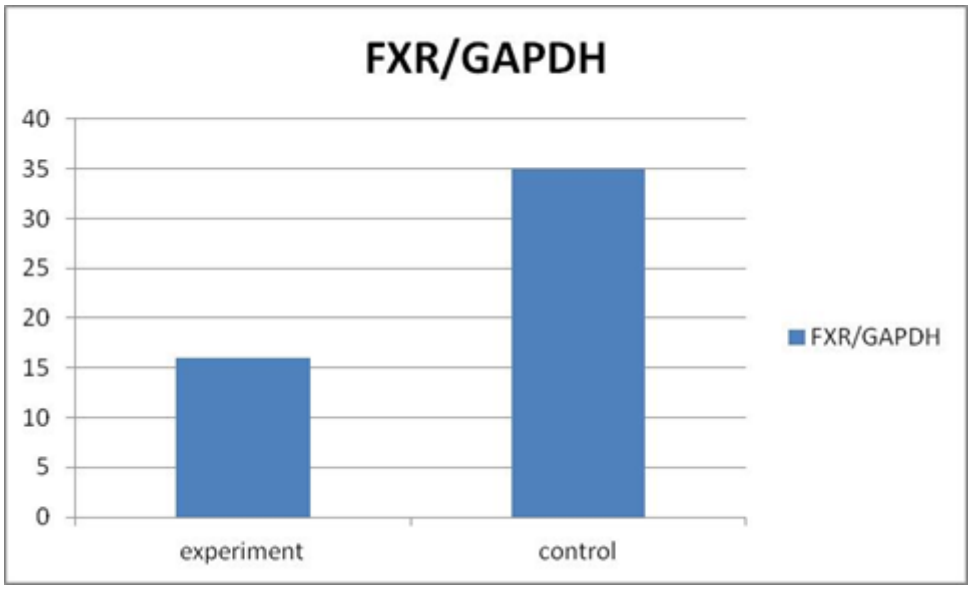

Figure 2

Analysis of FXR mRNA expression. In hilar cholangiocarcinoma and normal hilar bile duct tissues, after eight cycles, the FXR/Gapdh ratios were 16 and 35, respectively ($t = 2.392$, $P < 0.05$).

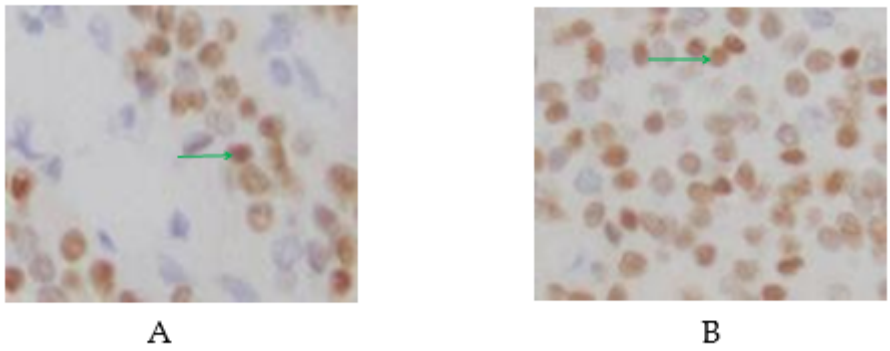

Figure 3

Analysis of FXR expression. A) (×200) FXR expression in Tumor of an experimental rat. B) (×200) FXR expression in normal hilar bile duct tissue from a control rat. The green arrows indicate FXR.

Supplementary Files

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