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Research Article

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Expressions of Orphan Nuclear Receptor TR3/Nur77 in Chronic Hepatopathy and Its Clinical Significance

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Abstract

Objective: Although great success has been achieved in cancer treatment, current cancer therapies, including anti-tumorigenesis and anti-angiogenesis, still face the problems of insufficient efficacy, resistance and intrinsic refractoriness, in addition to their toxic side effects. There is a demand to identify additional targets that can be blocked to turn off the downstream effects of most, if not all, pathways. Our studies suggest that orphan nuclear receptor TR3 (human) / Nur77 (mouse) is such a target. Most recently, we reported that TR3/Nur77 expression in human hepatic cancer tissues correlates well with tumor progress, suggesting that TR3 is a specific therapeutic target for hepatic cancers. However, the correlation of TR3/Nur77 expression in hepatocellular carcinoma (HCC) with chronic hepatitis has not been studied.

Methods: The expression of TR3/Nur77 was analyzed in human primary hepatic cancer specimens from patients that have complete medical records with Immunohistochemically staining. The statistical analysis was used to access the significance of TR3 expression in tumor tissues, cirrhosis tissues and chronic hepatitis tissues with and without hepatitis B virus infection (HBV(+) and HBV(-)), which were obtained from para-tumor tissues.

Results: The positive rates of TR3 / Nur77 expression in hepatocellular carcinoma, cancerous liver cirrhosis and chronic hepatitis are 66.67%, 30%, and 20%, respectively, which are statistic significant ($p<0.05$). The positive rates of TR3 / Nur77 expression in hepatocellular carcinoma are statistic significant ($p<0.05$) with 81.25% and 20% in HBV (+) or HBV (-), respectively.

Conclusion: The positive expression rate of TR3 / Nur77 in hepatocellular carcinoma is higher than that in chronic hepatitis and cirrhosis. The positive rate of TR3 / Nur77 expression in hepatocellular carcinoma is higher with HBV infection than that without infection. Our results suggest that TR3/Nur77 plays an important role in the progression of chronic hepatitis, and the occurrence and development of HCC.

Keywords

Hepatocellular carcinoma; Nuclear orphan receptor TR3; Nur77; Chronic hepatitis; Cirrhosis; Hepatitis B virus (HBV)

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Abbreviations: VEGF-A: Vascular Endothelial Growth Factor-A; HBV: Hepatitis B Virus; HCC: Hepatocellular Carcinoma

Introduction

We were the first to identify that TR3/Nur77, a member of nuclear receptor IV subfamily of transcription factors [1], is a critical mediator of angiogenesis [2-4]. TR3/Nur77 is highly and transiently up-regulated by angiogenic factors vascular endothelial growth factor-A (VEGF-A), histamine and serotonin, but not by basic fibroblast growth factor (bFGF), placental growth factor (PlGF) and platelet-derived growth factor (PDGF) in cultured endothelial cells (EC) and during angiogenesis *in vivo* [2-4]. Endothelial cell proliferation, migration and tube formation in vitro and angiogenesis and microvessel permeability in vivo, induced by VEGF-A, histamine or serotonin, and tumor growth are inhibited by TR3 antisense DNA or shRNA in vitro and in Nur77 knockout mice, respectively [2-4]. Overexpression of TR3/Nur77 is sufficient to induce endothelial cell proliferation, migration and tube formation *in vitro,* angiogenesis, microvessel permeability and normal skin wound healing in our transgenic mice, in which, Nur77 full-length cDNA is inducibly and specifically expressed in mouse endothelium (EC-Nur77-S transgenic mice), respectively [2-4]. However, both the Nur77 null mice and EC-Nur77-S transgenic mice are healthy [5,6]. Therefore, TR3/Nur77 is an excellent target for pro-angiogenesis and anti-angiogenesis therapies.

It was also reported that TR3/Nur77 plays important roles in carcinogenesis, apoptosis [5], brown fat thermogenesis [7,8], inflammation, metabolism diseases, stress and addiction (reviewed in [9-12]). However, the clinical correlation of TR3 expression in human cancers is understudied. Hepatocellular carcinoma (HCC) that accounts for 80% of all liver cancers worldwide [13], is the sixth most common neoplasm and the third most frequent cause of cancer death [14]. In the clinic, most HCC are diagnosed in advanced stage without effective treatment [13]. Most recently, we reported that the expression of TR3 in human hepatic cancer tissues correlates very well with tumour progress and metastasis [15]. We found that TR3 is highly expressed in human hepatic cancer tissues, but not in human normal liver tissues. The positive expression yields of TR3 are significantly different among cancer tissues, para-cancer tissues, and normal liver tissues. Further, the expression of TR3 is significantly higher in cancer tissues than that in para-cancer tissues and in normal tissues. The levels of TR3 expression in human hepatic cancer tissues correlates well with tumors that are at low/middle degree of tumor differentiation and have portal vein thrombosis, metastasis and recurrence, but not with age, gender, tumor number and Alpha-fetalprotein (AFP) volume [15]. The results suggest that TR3 is a novel target for human cancer therapy. However, the correlation of TR3/Nur77 expression in HCC with chronic hepatitis has not been studied. This study is to investigate the expression of TR3 / Nur77 in liver tissue of patients with chronic hepatitis, cirrhosis and liver cancer, and to understand its relationship with the progression of chronic hepatitis. We find that the positive rates of TR3 / Nur77 expression in hepatocellular carcinoma, cancerous liver cirrhosis and chronic hepatitis are 66.67%, 30%, and 20%, respectively,

which are statistic significant ($p<0.05$). The positive expression rate of TR3 / Nur77 in hepatocellular carcinoma is higher than that in chronic hepatitis and cirrhosis. The positive rate of TR3 / Nur77 expression in hepatocellular carcinoma is higher with HBV infection than that without infection. Our results suggest that TR3/Nur77 plays an important role in the progression of chronic hepatitis, the occurrence and development of HCC.

Material and Methods

Patient material and tissue

The paraffin-embedded postoperative tissue specimens classified by pathological analysis were obtained from the archives of Department of Pathology, the Second Affiliated Hospital of Guangzhou Medical University, P. R. China, between January to December 2013. Twenty-one human primary liver cancer specimens from patients that have complete medical records were retrieved. Approval of the current project was obtained from the local ethics committee.

The main characteristics of 21 patients were summarized in our most recent publication [15]. Ages of all patients in this study are between 32 and 70 years (median, 58 years), with 13 cases of males and 8 cases of females. There are 8 cases and 13 cases with the tumor diameter less/equal or greater than 5 cm, respectively. Histological analysis indicated that 13 cases and 8 cases belong to low/middle and highly differentiated tumors, respectively. Among the 21 patients, 14 and 16 patients complicated with portal vein thrombosis and infection with HBV, respectively. There are 20 cirrhosis tissues and 15 chronic hepatitis tissues obtained from para-tumor tissues, respectively.

Compliance with ethical standards

The research involves human samples. The study was approved by the Ethics Committee of The Second Affiliated Hospital of Guangzhou Medical University.

Tissue processing and immunohistochemically staining

All tissues were fixed with 10% formalin, embedded with paraffin. Immunochemical staining on 4 m sections was performed with an antibody against TR3/Nur77 (Univ-bio, Shanghai, CHINA), using immunological staining reagents following the protocol provided by the manufacture (Univ-bio, Shanghai, CHINA). PBS buffer was used as negative control.

Data analysis

Stained sections were analysed by two independent pathologists. At least 10 fields with 400x amplification from each stained section were randomly chosen to be photographed with Olympus BX41 microscope (Olympus Scientific Solutions Americas Inc. Waltham, MA). Brown staining is referred as positive. Using half-quantitative measurement, the staining intensity was recorded as two levels, 1 as no staining and 2 as positive staining. The positive percentage of cancer cells was counted as 3 levels, 1 as ≤ 25%, 2 as 26%～75%and 3 as ≥ 76%. The final score for each section was the multiplication of these two scores (1~6). The sections were referred to be positive and negative with final score ≥ 3 and <3, respectively.

Statistics

Chi-square test and Fisher exact test were used for statistical analysis. A level of $p<0.05$ is considered significant.

Results

Expression of TR3 / Nur77 in chronic hepatitis, cirrhosis and hepatocellular carcinoma

To study the correlation of TR3/Nur77 expression in HCC with chronic hepatitis, we examined human normal liver tissues and hepatic cancer tissues by immunostaining with an antibody against TR3/Nur77. The stained sections were analyzed by two independent pathologists. At least 10 fields with 400x amplification were randomly chosen from each stained section. Using half-quantitative measurement, the staining intensity was recorded as two levels, 1 as no staining and 2 as positive staining. The positive percentage of cells was counted as 3 levels, 1 as ≤ 25%, 2 as 26%～75% and 3 as ≥ 76%. The final score for each specimen was the multiplication of these two scores. They were referred to as high and low with final score ≥ 3 and < 3, respectively. The positive expression yields of TR3 are 20% (3/15), 30% (6/20) and 66.67% (14/21) in chronic hepatitis tissues, liver cirrhosis tissues and hepatocellular carcinoma tissues, respectively, which are statistic significant ($\chi2=9.453$, $p<0.05$). The expression of TR3 is significantly higher in hepatocellular carcinoma than that in chronic hepatitis ($p=0.0073$) and cirrhosis ($\chi2=5.647$, $p<0.05$) (Table 1). The data indicate that the positive rate of TR3/Nur77 expression in hepatocellular carcinoma is higher than that in both chronic hepatitis and cirrhosis.

Correlation of TR3 / Nur77 expression with HBV infection

To study whether TR3 expression correlates with HBV infection, we analyzed tissues obtained from patients with and without HBV infection. The positive rates of TR3 / Nur77 expression are 81.25% (13/16) and 20% (1/5) in patients with and without HBV infection, respectively, which are statistic significant ($p<0.05$) (Table 2). Our data indicate that positive rate of TR3 / Nur77 expression in hepatocellular carcinoma infected with HBV infection is higher than that in hepatocellular carcinoma without infection.

Conclusions

Most recently, we reported that the expression of TR3 in human hepatic cancer tissues correlates very well with tumor progress and metastasis [15]. Here, we find that the positive rates of TR3 / Nur77 expression in hepatocellular carcinoma, cancerous liver cirrhosis and chronic hepatitis are 66.67%, 30%, and 20%, respectively, which are statistic significant ($p<0.05$). The positive expression rates of TR3 / Nur77 in hepatocellular carcinoma with HBV (+) or HBV (-) are 81.25% and 20%, respectively, which are statistic significant ($p<0.05$).

Angiogenesis is critical for tumor growth [16-20]. One of the most important angiogenic factors is vascular endothelial growth factor-A (VEGF-A) which is widely studied in the angiogenesis and progression of hepatocellular carcinoma [20]. Nuclear orphan receptor TR3 / Nur77 is a major transcription factor downstream of VEGF-A, histamine and serotonin, controlling pathologic angiogenesis [2-4]. TR3 / Nur77 are highly and transiently regulated in VEGF-A-induced endothelial cells and several types of pathological changes [2-4]. The results of this study show that the positive expression rate of TR3 / Nur77 in 21 cases of HCC is 66.67%, which was significantly higher than that in chronic hepatitis and cirrhotic tissues. Our results are consistent with the report from Kanematsu et al. who found that the expression of VEGF receptor in hepatocellular carcinoma is 7 times and significantly higher than that in normal liver tissue and in adjacent liver cancer tissues, respectively [21]. Expression of TR3/

Table 1: Expression of TR3 / Nur77 in hepatocellular carcinoma of chronic hepatitis cirrhosis.

Tissues	No. of patients	Positive expression rate	χ2	P
Cancer tissues	21	14(66.67%)	9.453	<0.05
cirrhosis	20	6 (30%)*		
chronic hepatitis	15	3 (20%)**		

Note: *Cancer tissues compared with para-cancerous hepatic cirrhosis, $\chi 2=5.647$, $p<0.05$. **Cancer tissues compared with chronic hepatitis tissue, $p<0.05$.

Table 2: Correlation of TR3 / Nur77 expression with HBV infection.

Tissues	No. of all tissues	No. of positive tissues	No. of negative tissues	Positive expression rate	p
HBV(+)	16	13	3	81.25%	0.025
HBV(-)	5	1	4	20%	

Nur77 correlates very well with the characteristics of hepatic tumors, including highly metastasized, early and easy recurrence and other clinical biological characteristics, suggesting that TR3 / Nur77 plays an important role in the progress of chronic hepatitis to hepatic cirrhosis and hepatic cancer.

It is well known that chronic hepatitis, in which, HBV infection is the main cause, is a prelude to cirrhosis and hepatic cancer. HBV infection is closely related to the occurrence and development of primary hepatic cancer. HBsAg-positive HCC patients account for 70% to 90% of the total number of chronic hepatitis. HBV-related hepatocellular carcinoma has a lower degree of differentiation and poorer prognosis than non-HBV-infected hepatocellular carcinoma [22]. In the process of chronic hepatitis and cirrhosis, the repeated alternation of liver cell degeneration, necrosis, fibrous hyperplasia, and hepatocellular nodular regeneration results in liver fake lobular formation. This blood circulation disorders and local liver fiber / sclerosis inevitably cause hypoxia. Hypoxia activates hepatocytes and hepatic stellate cells, through autocrine and paracrine way, to induce VEGF-A and other cytokines [23]. It was reported that HBV infection up-regulates the expression of VEGF-A in hepatic cancer to induce tumor angiogenesis [24]. We find that the positive rate of TR3 / Nur77 expression in HBV infection group is significantly higher than that in non-HBV infection group, suggesting that HBV infection up-regulates the expression of TR3 / Nur77 in HCC tissues. HBV-infected hepatocellular carcinomas have its own unique biological characteristics. The expression of TR3 / Nur77 in these patients is significantly higher than that of non-HBV-infected hepatocellular carcinoma, which may be one of the reasons for the poor prognosis of HBV infection.

In summary, we find that the positive expression rate of TR3 / Nur77 in hepatocellular carcinoma is significantly higher than that in chronic hepatitis and cirrhotic tissues. The positive rate of TR3 / Nur77 expression in HBV (+) hepatic cancer tissues is significantly higher than that in non-HBV infected tissues, suggesting that TR3 / Nur77 plays an important role in the progression of chronic hepatitis, cirrhosis and the occurrence and development of hepatic cancer.

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