UDC 616.36-002.12-036.12-097

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THE ROLE OF SIGNALING PATHWAYS IN IMMUNOPATHOGENESIS OF CHRONIC VIRAL HEPATITIS

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Introduction

The widespread prevalence of viral hepatitis, the ability of HCV and HBV to long-term persistence, fibrotic changes and cirrhosis of the liver, the possible development of hepatocarcinoma necessitate further studies of the regulation mechanisms of immune homeostasis, as well as factors determining the interaction of the pathogen and the macroorganism.

A number of authors have shown that the relationship of pathogenetic mechanisms and outcomes of chronic viral hepatitis determines the ratio of responses of innate and adaptive immunity [1–3].

It is known that a prerequisite for the differentiation of lymphocytes into effector cells is their transition from a resting state to the cell cycle [4]. Several signaling pathways usually turn on in the cell, each of which ends with the formation of a transcription factor [4–6].

In the pathogenesis of many liver diseases, signaling pathways for regulating the passage of intracellular signals are of great importance. These include the nucleous factor NF-κB, which is involved in the regulation of transcription of certain genes that control immune and inflammatory responses. In the liver, the nuclear factor NF-κB is involved in the regulation of inflammatory responses, as well as in carcinogenesis [7–9].

Interferon signaling pathways play a significant role in the pathogenesis of HCV infection. It has been established that in HCV infection, a number of stages of intracellular signaling cascades necessary for the production of IFN type I and the expression of the interferon-stimulated ISG gene are blocked [5; 10].

IFN also influences the post-transcriptional regulation of gene expression, inducing the synthesis of initiation and elongation factors of eIF2a, eIF2b and others [11]. Individual viral proteins are of particular importance in the induction of immune disorders that contribute to the long-term persistence of hepatitis C.

A nucleocapsid protein (core) provides a signal for inducing cell apoptosis by activating a mitochondrial apoptotic signal [12]. Non-structural proteins NS3 and NS5A HCV affect the signaling pathways of interferon in the affected cell [13].

It has been established that microRNAs as gene expression regulators play a significant role in the mechanisms of the immune response in infectious pathologies. MicroRNAs are involved in the regulation of TLR signaling pathways, their signaling proteins, transcription factors, cytokines, thereby changing the reactions of innate immunity [14; 15].

MicroRNA 122 is identified in hepatocytes and is expressed at a rather high level and is able to bind to RNA of the HCV genome and protect the virus RNA from degradation by hepatocyte exoribonucleases [16; 17].

It was found that microRNA 132 expression level decreases in hepatocellular carcinoma in patients infected with hepatitis B virus, which causes activation of the B-signaling kinase/protein kinase that stimulates the development of hepatocellular carcinoma [18]. The experiment showed that for various infectious and inflammatory diseases, for example, for HBV infection, there is an imbal-

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ance of microRNA 125 and microRNA 164 [14].

In the maintenance of homeostasis in the liver pathology, one of the key signaling pathways for regulating the passage of intracellular signals is the BMP/SMAD pathway.

It was established that SMAD proteins are involved in the transmission of signals obtained through the cell surface receptors of various proteins of the TGF family. After activation, these proteins move to the nucleus, where they can activate transcription [19].

It was shown that the SMAD7 protein was involved not only in the regulation of cell signaling, differentiation and apoptosis of cells, but also was the most important antagonist of TGF β [20].

SMAD6 and SMAD7 are a part of a group of proteins that block the receptor of mediated phosphorylation of R-SMAD proteins. Moreover, the initiation of tyrosine kinase transcription can be blocked by SMAD7 [19; 21].

The modulation of the BMP/SMAD signaling pathway is considered as a possible therapeutic approach to the treatment of chronic viral hepatitis [22].

The aim of the study was to evaluate the association of SMAD7 gene polymorphism with morphological changes in the liver in chronic viral hepatitis of various etiologies.

Materials and methods

31 patients with mixed hepatitis with chronic hepatitis (B + C) were examined in the study, the age of the patients ranged from 29–66 years. All patients participating in the examination were registered in the clinic "Odessa City Clinical Infectious Disease Hospital". In the group of patients studied, men predominated (23 people), there were only 8 women. Patients with HIV or/and other hepatotropic viruses were excluded from the study.

Table 1

Characteristics of the studied polymorphisms

Gene	SMAD family member 7
Polymorphism	(SMAD7 C>T)
Primer name	rs4939827
Nucleotide sequence	TGTCTCTAATCCACCATGCTCAC

To compare patients and healthy individuals, a control group of 30 practically healthy middle-aged individuals was formed. The number of men and women (15 people each) was the same.

Confirmation of the etiology of the disease was carried out by determining specific serological markers HCV (aHCV common, aHCV-IgM, aHCV IgG) and HBV markers (HBsAg, HBeAg) by enzyme-linked immunosorbent assay using DiProfMed test systems. Molecular biological studies have included the determination of HCV RNA and HBV DNA by a qualitative and quantitative method using a polymerase chain reaction (Abbott, Real-time test system and CFX96 Detector Amplifier, BioRad). The studies were performed at the Virology Laboratory of the Odessa City Clinical Hospital and the SINEVO Laboratory.

Molecular genetic studies included the determination of polymorphic variants of the gene SMAD family member 7 (SMAD7 C>T). DNA was isolated using the DNA-EXPRESS blood kit (NPF Litekh, RF). Polymorphism was studied by amplification of the corresponding sections of the genome by PCR. The structure of the primers used and the parameters of the temperature cycles is described in the literature and the genomic database.

Testing technique of allelic polymorphism of genes in the framework of a pilot project and the general characteristics of the non-invasive method for evaluating Fibrotest liver fibrosis were described in our previous works [23].

To identify correlations between individual indices, the Spearman correlation coefficient was used.

Results

The analysis of the frequency of occurrence of different variants of the allelic polymorphism of the SMAD7 gene in patients with chronic hepatitis C and chronic hepatitis B is presented in our previous works [24].

The analysis of the frequency of occurrence of allelic polymorphisms of the SMAD7 gene in patients with mixed hepatitis chronic hepatitis (B + C) revealed the following (Fig. 1).

In patients with chronic hepatitis B + C, the homozygous CC

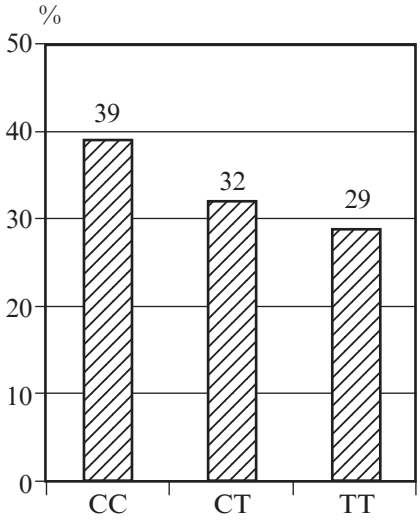

Fig. 1. Frequency distribution of the genotypes SMAD family member 7 (SMAD7 C>T) in patients with chronic hepatitis B + C

genotype SMAD family member 7 (SMAD7 C>T) prevailed — 39%. The number of carriers of the heterozygous genotype CT SMAD family member 7 (SMAD7 C>T) were slightly fewer — 32%. There were fewer carriers of the mutant homozygous genotype SMAD family member 7 (SMAD7 C>T) than the remaining genotypes — 29%. No significant difference in indices was found. The frequency of the C allele was 0.53, and the frequency of the T allele was 0.46.

To identify the relationship between the degree of liver fibrosis and certain genotypes of SMAD family member 7 (SMAD7 C>T), all patients were divided into 3 groups in accordance with the degree of fibrosis (Fig. 2).

In the group of patients with chronic hepatitis C, patients with a minimal degree of fibrosis (F0–F1) prevailed — 46%. In the group of patients with chronic hepatitis B + C, the number of patients with a minimum degree of fibrosis (F0–F1) and a maximum degree of fibrosis (F3) was the same — 42% each.

The relationship between the degree of liver fibrosis and allelic polymorphism of the genes, as well as the relationship between the individual cytokine genotypes, was evaluated using Spearman's correlation coefficient (Table 2, 3).

In patients with chronic hepatitis C, a direct moderate correlation between the degree of fibrosis and the SMAD family member 7 genotype was revealed: a lower degree of fibrosis was observed in the carriers of the SS genotype, a higher degree of fibrosis was in the carriers of the TT genotype.

In patients with chronic hepatitis B + C, an inverse strong correlation was found between the degree of fibrosis and the SMAD family member 7 genotype: a lower degree of fibrosis was observed in the carriers of the TT genotype, and a higher degree of fibrosis was observed in the carriers of the CC genotype.

As a result of the pilot studies, it can be assumed that the genetic component of severe fibrosis in patients with hepatitis of various etiologies is not significantly different: in all studied groups, the degree of F3 fibrosis is associated with the with CT allele SMAD family member 7. This index can be used as an additional criterion in the diagnosis and prognosis of the chronic liver disease.

Ключові слова: хронічний гепатит С, хронічний гепатит В, хронічний гепатит В + С, family members factor SMAD 7.

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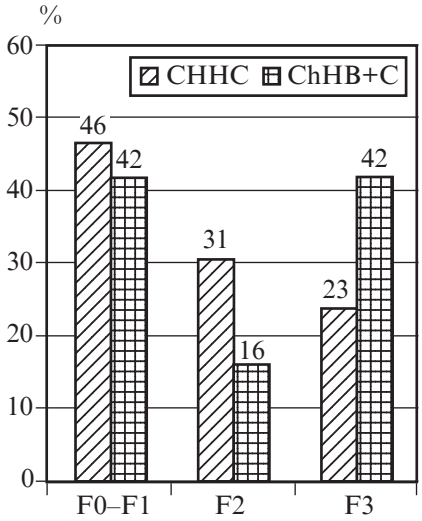

Fig. 2. Percentage of patients with chronic hepatitis C and chronic hepatitis B + C with varying degrees of fibrosis

Table 2

Correlation coefficients between the degree of fibrosis and the genotype of SMAD family member 7 (SMAD7 C>T) in patients with chronic hepatitis C

Correlation coefficient	SMAD family member 7	F
SMAD family member 7	1	0.418**
F	0.418**	1

Note. In table 2, 3: ** — $p<0.05$.

Table 3

Correlation coefficients between the degree of fibrosis and the genotype of SMAD family member 7 (SMAD7 C>T) in patients with chronic hepatitis B + C

Correlation coefficient	SMAD family member 7	F
SMAD family member 7	1	-0.853**
F	-0.853**	1

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Submitted 10.09.2019
Reviewer ass. prof. A. I. Ponomarenko, date of review 5.11.2019

UDC 616.36-002.12-036.12-097
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РОЛЬ СИГНАЛЬНИХ ШЛЯХІВ У ПАТОГЕНЕЗІ HCV-ІНФЕКЦІЇ

Метою дослідження було оцінити зв'язок поліморфізму гена *SMAD7* з морфологічними змінами печінки при хронічному вірусному гепатиті різної етіології.

Матеріали та методи. У дослідженні взяли участь 31 пацієнт з хронічним гепатитом змішаної етіології (В + С). Молекулярно-генетичні дослідження включали визначення поліморфних варіантів гена family members factor SMAD7 *(SMAD7 C>T)*.

Результати. У результаті пілотних досліджень можна припустити, що генетичний компонент тяжкого фіброзу у пацієнтів з гепатитом різної етіології суттєво не відрізняється: у всіх досліджуваних групах ступінь фіброзу F3 асоціюється із CT алелем family members factor SMAD7. Цей показник може бути використаний як додатковий критерій діагностики та прогнозу хронічних захворювань печінки.

Ключові слова: хронічний гепатит С, хронічний гепатит В, хронічний гепатит В + С, family members factor SMAD7.

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The aim of the study was to evaluate the association of *SMAD7* gene polymorphism with morphological changes in the liver in chronic viral hepatitis of various etiologies.

The study involved 31 patients with chronic hepatitis of mixed etiology (B + C). Molecular genetic studies included the determination of polymorphic variants of the gene SMAD family member 7 (SMAD7 C>T).

As a result of the pilot studies, it can be assumed that the genetic component of severe fibrosis in patients with hepatitis of various etiologies is not significantly different: in all groups under study, the degree of F3 fibrosis is associated with CT allele SMAD family member 7. This index can be used as an additional criterion in the diagnosis and prognosis of chronic liver disease.

Key words: chronic hepatitis C, chronic hepatitis B, chronic hepatitis B + C, family members factor SMAD7.

УДК 616.853-092.9

М. П. Первак

ОСОБЛИВОСТІ МАКСИМАЛЬНИХ ЕЛЕКТРОШОКОВИХ СУДОМ НА ТЛІ ТРАНСКРАНІАЛЬНОГО ВПЛИВУ АНОДОМ І КАТОДОМ ПОСТІЙНОГО СТРУМУ НА МОЗОЧОК У ЩУРІВ

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Вступ

Установлено протисудомний вплив транскраніального подразнення постійним струмом (ТППС) структур мозочка на прояви пентиленететразоліндукованих кіндлінгових судом [2; 3]. Визначено, що ефект запобігання судомам реєструвався на тлі попереднього ТППС катодом постійного струму паралеоцеребелярної кори і забезпечувався застосуванням блокаторів рецепторів, які активує пероксисомний проліфератор (PPARγ) [3].

Однак залишаються мало вивченими як ефективність за-

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