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POLYMORPHISM OF SMAD7 (RS4939827) AND EIF3H (RS16892766) GENES AS A CRITERIUM OF FIBROSIS PROGRESSION RATE IN PATIENTS WITH CHRONIC HEPATITIS C AND B

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The purpose of the study was to assess the possibility of the relationship between the gene polymorphism of SMAD family member 7 (SMAD7C> T), the eukaryotic translation initiation factor (eIF3hC> A) and the degree of liver fibrosis in a pilot project in patients with chronic hepatitis B and chronic hepatitis C. Differences in the frequencies of alleles of the SMAD7 gene in the ethnically homogeneous group of residents of the Odessa region in patients with chronic hepatitis C and chronic hepatitis B compared to healthy individuals ($p < 0.05$) were found. The presence of correlations was established: in patients with chronic hepatitis C, there is a direct weak correlation between the degree of fibrosis and SMAD7 genotypes ($p < 0.01$); in patients with chronic hepatitis B, there is a direct strong correlation between the fibrosis degree and SMAD7 genotypes ($p < 0.01$). The correlation between the degree of liver fibrosis and certain genotypes of SMAD7 in patients with chronic hepatitis C and chronic hepatitis B permits to use the information obtained as one of the criteria for the rate of fibrotic processes progression in the liver.

Key words: SMAD family member 7, eukaryotic translation initiation factor eIF3h, chronic hepatitis C, chronic hepatitis B.

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ПОЛІМОРФІЗМ ГЕНІВ SMAD7 (RS4939827) І EIF3H (RS16892766) ЯК КРИТЕРІЙ ШВИДКОСТІ ФІБРОЗУ У ХВОРИХ НА ХРОНІЧНІ ГЕПАТИТИ С І В

У статті вивчено взаємозв'язок між поліморфізмом генів SMAD7, eIF3h і ступенем фіброзу печінки в пілотному проекті у хворих на хронічний гепатит С та хронічний гепатит В. Встановлено відмінності по частотах алелей гена SMAD7 у хворих на хронічний гепатит С та хронічний гепатит В у порівнянні з практично здоровими особами. Виявлено кореляційні зв'язки: прямий слабкий у хворих на хронічний гепатит С між ступенем фіброзу і генотипами SMAD7; у хворих на хронічний гепатит В – прямий сильний між ступенем фіброзу і генотипами SMAD7. Наявність взаємозв'язку ступеня фіброзу печінки і певних генотипів SMAD7 у хворих на хронічний гепатит С та хронічний гепатит В дозволяє використовувати отриману інформацію як один із критеріїв швидкості прогресування фібротичних процесів печінки.

Ключові слова: SMAD family member 7, еукаріотичний фактор ініціації трансляції eIF3h, хронічний гепатит С, хронічний гепатит В.

The work is a fragment of the research project "Pathogenetic significance of disorders in the lipid peroxidation – antioxidant system (LPO / AOS) in patients with viral infections of various etiologies", state registration No. 0118U007316.

Currently, molecular genetic studies in patients with chronic viral hepatitis are aimed at developing predictive models of both the risk of developing liver fibrosis and that of hepatocarcinoma in a particular patient based on the model of intergenic interactions [1, 3, 15, 5].

SMAD – proteins have been identified as mediators of transcriptional activation. SMAD – proteins are involved in the transmission of signals received through the cell surface receptors of various proteins family members associated with TGFβ. After activation, these proteins move to the nucleus, where they can activate transcription [11, 13].

Several studies have shown that the SMAD7 protein is involved in the regulation of cell signaling, proliferation, differentiation, and apoptosis of cells, and is the most important antagonist of TGFβ [6, 10].

It has been established that the initiation of transcription depends on tyrosine kinase and can be blocked by SMAD7. SMAD6 and SMAD7 belong to a group of proteins that block the receptor of mediated R-SMAD proteins phosphorylation. It was experimentally established that SMAD7 prevents the activation of Ito (stellate) cells and liver fibrosis, which may contribute to inhibition of the processes of malignant transformation and metastasis [7, 8].

There is evidence in the literature that some of the SMAD proteins are involved in the regulation of microRNA biogenesis. New biological markers for predicting the course and outcomes of various pathological processes, including those in infectious pathology, are being studied. MicroRNAs are considered as such markers. Micro RNAs block protein translation and induce degradation of translational risk RNA (mRNA), regulate post-transcriptional gene expression [9, 4].

It was shown that among numerous microRNAs in hepatocytes, microRNA-122 was identified, where it is expressed at a high level. At the same time, microRNA-122, as one of the most important

regulators of gene expression, interacts with the untranslated region of the 5 'ITR RNA of the HCV genome, which protects the viral RNA from degradation by exoribonucleases of hepatocytes [12, 14, 15].

The search for molecular markers and early diagnosis of hepatocarcinoma continues to be a topical field of molecular genetic research. Highly specific and highly sensitive markers of hepatocellular carcinoma have not been established yet. However, a number of avenues are being considered to identify potential markers of hepatocellular carcinoma.

Various approaches are considered as promising biomarkers for predicting the course and outcomes of chronic hepatitis of viral etiology: transforming growth factor B1 (TGF-β1), microRNA, determination of mutations in intracellular signaling molecules – the factor of eukaryotic translation initiation, the role of the phosphorylated eIF3h subunit in carcinogenesis and other processes [3, 11, 6].

Taking into account the specific features of the initiation of translation in RNA of the hepatitis C virus, further study on the interaction of the BMP/SMAD7 signaling pathway and signaling pathways for the regulation of protein synthesis at the stage of initiation of metabolic and signaling pathways of transduction in eukaryotes creates prospects in future to reduce the risk of developing cirrhosis through the BMP/SMAD7 signaling pathway.

The purpose of the study was to assess the possibility of a correlation between the polymorphism of the SMAD family member 7 (SMAD7C> T) genes and eukaryotic translation initiation factor (eIF3hC> A) and the degree of liver fibrosis in the pilot project in patients with chronic hepatitis B and chronic hepatitis C living in the Odessa region, and to assess the prospects for predicting the outcome of chronic viral hepatitis based on these genetic markers.

Materials and methods. The study included 41 patients with chronic hepatitis B and 100 patients with chronic hepatitis C aged 62 to 18 years. Women were 47% (66 persons), and men - 53% (75 persons), all of them being residents of the Odessa region. Patients included in the study were selected using random sampling technique. The duration of the disease did not exceed 10 years. All patients included in the study signed an informed voluntary consent. The methodology of this clinical observation meets the requirements of the ONMedU Bioethics Committee (Protocol No. 179 of November 19, 2010).

Examination was carried out on an outpatient basis at the hepatological center of the KNP "Odessa City Infectious Diseases Hospital". To establish the final diagnosis, the routine biochemical tests were used (an increase in the activity of AST and ALT, the concentration of total bilirubin and its fractions). Serological markers of parenteral hepatitis (antigens HBeAg and HBsAg, antibodies aHCV) were determined by ELISA, quantitative and qualitative content of HCV RNA and HBV DNA was determined by PCR.

The control group is represented by 39 practically healthy individuals, their mean age is 32±1.05 years. The number of women and men was practically the same (20 men and 19 women).

Allelic polymorphism of SMAD family member 7 (SMAD7 C> T) genes and eukaryotic translation initiation factor (eIF3hC> A) was studied at the research laboratory on the basis of the St. Paul German Diagnostic Center by PCR method. The parameters of the performed temperature cycles and the primers' structure are described in the GenBank genomic database.

To assess the severity of morphological changes (the degree of liver fibrosis), the Fibrotest method of non-invasive diagnostics was used, which is an alternative to puncture liver biopsy.

The strength of the identified associations was assessed in terms of the odds ratio (OR) and its 95% confidence interval (CI). The difference in the compared values was considered statistically significant at $p < 0.05$, provided that the 95% CI value for OR does not include 1. In order to identify correlations between individual indices, the Spearman correlation coefficient was applied.

Results of the study and their discussion. In the study of SMAD7 (SMAD7 C> T) allelic polymorphism, certain differences were revealed in the control group and the studied groups of patients (tables 1-3).

Table 1

Comparison of SMAD7 gene polymorphisms occurrence frequency in patients with CHB and in the control group

	CHB patients (n=41)	Control group (n=39)	OR	95 % CI	p
SMAD7 (SMAD7 C>T) rs4939827					
CC	16	21	0.549	0.226–1.334	> 0.5
CT	16	6	3.520	1.204–10.289	< 0.01
TT	9	12	0.633	0.232–1.728	> 0.5

The study of allelic polymorphism SMAD7 (SMAD7 C> T) revealed a significant predominance of the heterozygous CT genotype in the group of CHB patients (39%) in comparison with the control group 15% ($p < 0.01$). In the group of practically healthy individuals, a high frequency of homozygous CC (54%) and TT

(31%) genotypes was established, while no statistically significant difference was found.

Table 2

Comparison of SMAD7 gene polymorphisms occurrence frequency in CHC patients and in the control group

	CHC patients (n=100)	Control group (n=39)	OR	95 % CI	p
SMAD7 (SMAD7 C>T) rs4939827					
CC	21	21	0.228	0.103–0.503	< 0.01
CT	56	6	7.0	2.693–18.196	< 0.005
TT	23	12	0.421	0.295–1.532	> 0.5

When studying the allelic polymorphism SMAD7 (SMAD7 C> T) in CHC patients, a certain predominance of the heterozygous CT genotype (56%) was also found in comparison with the control group (15%) (p <0.005).

Table 3

Comparison of the SMAD7 gene polymorphisms occurrence frequency between groups of patients with CHC and CHB

	CHC patients (n=100)	CHB patients (n=41)	OR	95 % CI	p
SMAD7 (SMAD7 C>T) rs4939827					
CC	21	16	0.403	0.188–0.916	< 0.05
CT	56	16	1.989	0.947–4.174	< 0.05
TT	23	9	1.062	0.443–2.545	> 0.5

In the process of studying the SMAD7 (SMAD7 C> T) allelic polymorphism occurrence frequency in CHC patients, a slight predominance of the heterozygous CT genotype (56%) (p <0.05) in comparison with the group of CHB patients (39%) was found. When studying the homozygous CC genotype (21% in CHC patients and 39% in CHB patients) and mutant homozygous TT (23% in CHC patients and 22% in CHB patients) occurrence frequency, no statistically significant differences were found in the groups of CHB and CHC patients.

The study of allelic polymorphism of the eukaryotic factor of eIF3h translation initiation in the control groups and patients with CHC and CHB did not reveal any significant differences. The mutant homozygous AA eIF3h genotype (eIF3h C> A) prevailed in the group of healthy individuals (85%), in patients with CHB (80%), and in patients with CHC (88%). Heterozygous CA eIF3h genotype (eIF3h C> A) was detected in a smaller number of healthy individuals (15%), as well as in patients with chronic hepatitis (20% in CHB patients and 12% in CHC patients). The homozygous CC eIF3h genotype (eIF3h C> A) was not found either in healthy individuals or in patients with chronic hepatitis.

To assess the correlation between the degree of fibrosis and allelic polymorphism of the eIF3h and SMAD7 genes, all patients were divided into three subgroups depending on the degree of fibrosis. In both groups, patients with a minimum degree of activity (F0-F1) prevailed – 46% and 41%; the number of patients with CHC moderate fibrosis (F2) made 31%, and patients with CHB – 34%. There were 23% of CHC patients with severe liver fibrosis (F3), and 25% of CHB patients.

The correlation between hepatic tissue fibrosis and allelic polymorphism of the studied genotypes was assessed using the Spearman's rank correlation coefficient (table 4).

Table 4

Correlation links between different eIF3h and SMAD7 genotypes and the degree of liver fibrosis in patients with CHC and CHB

	SMAD 7	eukaryotic factor eIF3h	A	F
patients with chronic hepatitis C				
SMAD 7	1	-0.104	0.315**	0.393**
eukaryotic factor eIF3h	-0.104	1	-0.078	-0.017
A	0.315**	-0.078	1	0.695**
F	0.393**	-0.017	0.695**	1
patients with chronic hepatitis B				
SMAD 7	1	-0.132	0.786**	0.947**
eukaryotic factor eIF3h	-0.132	1	-0.173	-0.108
A	0.786**	-0.173	1	0.741**
F	0.947**	-0.108	0.741**	1

Note: ** – statistically significant correlation (p< 0.01)

The presence of the following correlations was established:

- in patients with CHC – a direct weak correlation between the degree of fibrosis and the SMAD7 genotypes, p <0.01 (a lesser degree of fibrosis is observed in carriers of the CC genotype, a greater degree

of fibrosis – in carriers of the TT genotype);

- in patients with CHB – a direct strong correlation between the degree of fibrosis and genotypes SMAD7, $p < 0.01$ (a lower degree of fibrosis is observed in carriers of the CC genotype, a greater degree of fibrosis – in carriers of the TT genotype).

The study of the allelic gene polymorphism correlation is a topical field in modern hepatology. The work by L.V. Moroz et al. showed the association of allelic polymorphism of cytokine genes and the severity of liver damage in patients with chronic hepatitis C [2, 3].

The study of SMAD7 and eIF3h polymorphism in patients with chronic liver pathology of viral etiology was the first to be carried out. The absence of a significant difference in the frequency of SMAD7 genotypes in patients with CHC and CHB may serve as confirmation of the importance of this particular component in the genetic profile of patients.

The presence of a correlation between the liver fibrosis degree and genotypes SMAD7 and eIF3h permits to use the information obtained as one of the possible criteria for the rate of fibrotic processes progression in the liver. We can assume the existence of protective and pro-fibrotic alleles of various genes, which is reflected in the works of other authors [2, 4]. Further study of allelic polymorphism of cytokine genes in patients with chronic viral liver pathology will permit to create a personalized approach to the treatment tactics of such patients.

Conclusions

1. Differences are established by allele frequencies of the SMAD7 gene in an ethnically homogeneous group of the Odessa region residents in patients with CHC and CHB compared to practically healthy individuals ($p < 0.05$), which suggests a certain correlation between the polymorphism of this gene and the formation of a chronic infectious process.

2. Patients with homozygous CC SMAD7 genotype have lesser fibrotic changes in the liver than carriers of homozygous TT genotype ($p < 0.01$).

Prospects of further research lie in the fact that the mutation of the eukaryotic translation initiation factor eIF3h genes in patients with chronic hepatitis is possibly associated not only with the formation of chronic pathology, but also with the development of hepatocellular carcinoma, which will be the subject of further research.

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