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Correlation between p53 expression and hepatocellular carcinoma differentiation grade

Relevance: Hepatocellular carcinoma (HCC) is one of the world's most common liver cancers and fatal malignant neoplasms. According to GLOBOCAN 2018, liver cancer ranks sixth in incidence (842,000 new cases) and fourth in mortality (782,000 deaths) globally. In Kazakhstan, there is an increase in HCC incidence from 879 cases in 2016 up to 984 cases in 2017, which amounted to 5.5 cases per 100 000 population. In 2017, the five-year survival rate was 23.7%.

The mutations in the p53 gene that encodes the p53 protein are most frequent in HCC (35.2%). A high level of nuclear expression of p53 and a decrease in apoptosis of tumor cells with a simultaneous increase in their proliferative activity, noted in HCC cells, are important for the prognosis of the disease. There is a correlation between poor prognosis, a decreased survival of patients with HCC, and high expression of the p53 oncoprotein in HCC.

U. Nzeako et al. have revealed a strong correlation of HCC histological differentiation with the patient survival rate. Therefore, a study of the correlation between p53 expression and HCC differentiation grade is very relevant.

The purpose of this study was to analyze the correlation between p53 expression and HCC differentiation grade.

Results: p53 was overexpressed in 18% of HCC cases; the expression was high in 62% and low in 20% of HCC cases. In well-differentiated HCC (G1), the p53-immunopositive nuclei expression was low in 10 cases and high in 2 cases. In moderately differentiated HCC (G2), p53 was highly expressed in 21 cases and overexpressed in 2 cases. In poorly-differentiated HCC (G3), p53 was overexpressed in 7 cases and highly expressed in 8 cases of HCC. The analysis of the correlation between p53 expression and HCC differentiation grade by Spearman showed a high correlation ($r=0.79$, $p<0.01$), evidencing a direct dependence of p53 expression level on HCC gradation.

Conclusion: HCC differentiation grade highly correlates with p53 expression level in immunopositive nuclei of HCC cells. The obtained data evidences a dependence between p53 expression level and HCC gradation.

Keywords: liver cancer, hepatocellular carcinoma (HCC), p53, differentiation grade, hepatocellular carcinoma grading.

Introduction: Hepatocellular carcinoma (HCC) is the most common primary malignant neoplasm of the liver (up to 95% of all primary malignant liver tumors), which is characterized by the late manifestation of the cancer process and low five-year survival in the majority of cases [1].

Recently, the HCC incidence is growing worldwide. Over 600,000 new HCC cases are registered in the world every year. According to GLOBOCAN, liver cancer ranked 6th in the world in 2018 by incidence (842,000 new cases) and 4th by mortality (782,000 fatal cases) [2, 3].

In Kazakhstan, HCC incidence had grown from 879 cases in 2016 to 984 cases in 2017, amounting to 5.5 cases per 100 000 population. In 2017, HCC mortality was 3.3 cases per 100 000 population (596 fatal cases), and the five-year survival was 23.7% [4, 5].

HCC develops as a result of the accumulation of genetic defects of different genesis both in mature hepatocytes and in stem cells. According to the whole-genome sequencing, the mutations in the p53 gene that encodes the p53 protein are most common in HCC (35.2%), followed by the mutations in the CTNNB1-gene that encodes β -catenin (15.9%) [6].

The most important biological characteristics of HCC that determine the disease prognosis are the tumor transformation of the hepatic cell genes, a distortion of balance between the apoptosis levels and tumor cells proliferation (high level of p53 nuclear expression, decline of the tumor cells apoptosis level and simultaneously – elevation of their proliferation level), and the

acquisition of invasive and metastatic features by these tumor cells [7].

There is also a correlation between poor prognosis, a decreased survival of HCC patients, and high expression of the p53 oncoprotein in HCC [8].

The p53 gene mutations prolong the half-life of the non-functioning protein p53, which accumulates in the nucleus [9] and can be detected by IHC studies.

Nzeako et al. revealed a strong correlation of HCC histological differentiation with the patient survival rate. Low-grade HCC (G1, G2) was associated with higher survival, while in high-grade HCC (G3, G4), oncological relapses were more frequent [10].

Materials and methods: In total, the study involved 50 patients (28 men and 22 women) aged 34 to 74 years (mean 49.7 ± 0.2 years) with liver cirrhosis and HCC who were admitted to Syzganov National Scientific Center of Surgery in 2015-2018. A total of 56 HCC nodes in the liver were revealed, sized 1 to 16.3 cm, 50 of them were examined. The HCC was nodular in 76% cases, and multinodular – in 24% cases. All the patients underwent surgical treatment (liver resection, hemihepatectomy of the right or left lobe, liver transplantation).

The postoperative liver tissue with HCC was studied histologically and immunohistochemically (IHC). In total, 50 samples of stained-glass preparations from 50 HCC nodes were prepared. The glass preparations were stained with hematoxylin-eosin by Van Gieson. For IHC studies, paraffin sections with HCC tissue were deparaf-

finized and rehydrated following a standard technique. Antigen unmasking was conducted in a PT Link Dako (USA). Staining with monoclonal mouse antibody to p53 human protein, clone DO-7 (Dako, Denmark) was carried out using a Bio-Optica slide master. The IHC reaction was visualized using a Reveal Polyvalent HRP-DAB Detection System. The markers' expression was assessed using a ZEISS AXIO Imager Z2 (Germany) microscope with an Axiocam 506 color camera and ZEISS ZEN Imaging Software.

HCC cell malignancy degree was assessed by Edmondson and Steiner: G1 grade is a well-differentiated HCC, G2 – a moderately-differentiated HCC, G3 – poorly-differentiated HCC, G4 – an undifferentiated HCC [11].

The nuclear expression of the p53 HCC cells was assessed by the special scale proposed by Jian-Liu *et al.* in 2004: the presence of up to 10% of immunopositive nuclei indicated a low p53-expression, 11 to 29% of immunopositive nuclei – high p53-expression, over 30% of immunopositive nuclei in HCC cells – overexpression.

The statistical analysis was conducted using the SPSS 13.0 (demo version) statistical software and Microsoft Excel (USA). The correlation between the p53 expression lev-

el and the HCC differentiation grade was determined by Spearman analysis. The values of $p \leq 0.05$ were considered statistically significant.

Results and Discussion: The cytological study and the assessment of HCC malignancy by Edmondson and Steiner revealed a well-differentiated HCC (G1) in 12 cases (24%), moderately-grade differentiated HCC (G2) in 23 cases (46%), and poorly-differentiated HCC (G3) in 15 cases (30%). No undifferentiated HCC (G4) was found in the study.

IHC test results showed the expression of p53-immunopositive nuclei in HCC cells on 4-50% of the glass area under study. p53 was overexpressed (32% to 50%, mean 39.7%) in 18% of HCC; the p53 was expression was high (12% to 29%, mean 20.7%) in 62% of HCC, and was low (4% to 9%, mean 6.2%) in 20% of HCC (table 1, figure 1).

Table 1 – p53 expression depending on the percentage of immunopositive nuclei in HCC cells (n=50)

p53 expression level	Number, n (%)	min-max p53 ex-pression, %	Mean p53 expres-sion, %
Overexpression	9 (18%)	32-50%	39.7%
High expression	31 (62%)	12-29%	20.7%
Low expression	10 (20%)	4-9%	6.2%

Figure 1 – Examples of overexpression (A), high expression (B), and low expression (C) of p53 in immunopositive nuclei of HCC cells

According to the analysis of p53 expression level depending on the HCC differentiation grade, in well-differentiated HCC (G1) the expression of p53-immunopositive nuclei was low in 10 cases (4% to 9% of the glass area under study) and high in 2 cases (12%, 15% of the glass area under study). In moderately- differentiated HCC (G2), the expres-

sion of p53 was high in 21 cases (12% to 28% of the glass area under study) and overexpressed in 2 cases (32% and 35% of the glass area under study). In poorly-differentiated HCC (G3), the p53 was overexpressed in 7 cases (31% to 50% of the glass area under study) and highly expressed in 8 cases (12% to 29% of the glass area under study) (table 2).

Table 2 – p53 expression depending on the HCC differentiation grade and the corresponding percentage of immunopositive nuclei in HCC cells (n = 50)

HCC grade / p53 expression	Overexpression, n=9		High expression, n=31		Low expression, n=10	
	Abs.	%	Abs.	%	Abs.	%
G1	-	-	2	12%, 15%	10	4-9%
G2	2	32%, 35%	21	12-28%	-	-
G3	7	31-50%	8	12-29%	-	-

A box plot diagram was prepared to visualize the distribution of the obtained p53 expression values among the three differentiation grades (G1, G2, G3) (figure 2). Axis Y showed the p53 expression in immunopositive nuclei of HCC cells (in %); axis X showed the HCC grading. The overshoot included only 1 out of 10 values in the Grade I, or 1 out of 50 values in the entire sample. In the Grade I, the lower quartile was at a 6% level, the upper quartile – at a 9% level, and the median p53 expression in immunopositive nuclei of HCC cells was 7.5%. In

the Grade II, the range was between 12% and 35%, with the lower quartile at a 16% level, the upper quartile – at a 24% level, and the median p53 expression of 22%. Grade III showed the widest value range from 12% to 50%, with the lower quartile at a 26% level, the upper quartile – at a 38% level, and the median p53 expression of 29%. Thus, the comparative analysis of medians in all three groups showed growth in median value with the differentiation (Grade I – 7.5%, Grade II – 22%, Grade III – 29%).

Figure 2 – p53 expression in immunopositive nuclei of HCC cells depending on the differentiation grade I, II, III (box plot diagram)

Spearman correlation analysis was carried out to determine the dependence of the p53 expression level from the HCC differentiation grade. The results showed a high correlative relationship ($r=0.79$, $p<0.01$), meaning a straight-line dependence of the p53 expression level from the HCC differentiation grade.

In a comparative study of p53 gene mutations and p53 expression in the HCC cells nuclei conducted by Hsia *et al.*, the IHC tests revealed a positive p53 expression above 50% in 94% cases in the presence of the p53 gene mutation (exon 5 (codon 175), exon 6 (codon 198), exon 7 (codon 249). The authors highlighted that most of the p53 gene mutations were registered in poorly-differentiated HCCs [12].

According to Niu *et al.*, a positive p53 and C-myc expression in HCC correlated with histological differentiation. Namely, p53 expression was much higher in poorly-differentiated HCCs vs. well-differentiated HCCs ($p<0.05$) [13].

Teramoto *et al.* reported that p53 overexpression was mostly observed in poorly-differentiated tumors ($p=0.01$), in tumors >5 cm ($p=0.05$) and in giant-cell tumors ($p=0.03$) [14].

D'Errico *et al.* [15] and El-Emshaty *et al.* [16] have revealed a high correlation between p53 expression level and HCC grading ($p<0.01$) and suggested that a mutated p53 gene influences the HCC progression by changing the differentiation degree, thus increasing the tumor malignancy.

Our analysis also revealed a high correlation ($r=0.79$, $p<0.01$) between p53 expression level in immunopositive nuclei of HCC cells and the HCC differentiation grade. p53 expression was low in well-differentiated HCCs, was high in moderately-differentiated HCCs, and p53 was overexpressed in poorly-differentiated HCCs.

Conclusion: The analysis showed a high correlation between p53 expression in immunopositive nuclei of HCC cells and HCC grading. The obtained results are important for the prognostics of this disease.

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