The study the role of different signaling pathways in the process of hepatocytes polyplodization in the cholestatic liver model

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Introduction. As it is known hypertrophy, cell proliferation and one of its form- polyploidization are important in the restoration of structure and function of the liver. Precondition for the last two processes is to enter to the mitotic cycle. Nowadays there are four main pathways that provide cells to enter to the cell cycle: ERK 1/2; JNK1 / 2/3; P38 and ERK5. Nevertheless, it is not clear yet, the processes are initiated by the same signaling pathways, or there are differences among pathway activation factors. The study of this issue is relevant because there is not yet known at which stage of cell cycle the cell gets the signal for blocking the cytokinesis and / or karocynesis (incomplete mitosis), resulting in the formation of polyploid cells.

The interest in this issue also led from the results of the study of cholestatic liver model. In particular it was shown that: 1. the amount of high-ploidy cells is significantly increased in the liver parenchyma of adult rats during 4 days from the common bile duct ligation; 2. High-mitotic activity of hepatocytes on the 4th day of operation, serves to form polyploid cells.

By considering all of this above, we decided the determination of the molecular mechanism of initiation of polyplopidation in cholestatic liver by selective blocking of the above mentioned 4signaling paths. Due to the fact that the leading role in proliferation is going to hepatocytes growth factor (HGF), and the concentration of HGF is increased on the second day after the common bile duct ligation, it was interesting to determine the role of HGF in the process of polyploidation in the cholesterol liver model. MEK 1 and MEK 2 are the key molecules of the signal pathway of ERK1/2.

Determination of their role in polyploidization is important, because they can be activated by avoiding HGF receptor - C-Met. Beside to ERK1 / 2, it was interesting to study a JNK signaling pathway in polyplodization. Proteins of JNK family are activated by the lack of cytokines, ultraviolet radiation, growth factors and by the activity of some G protein (G12/13) associated receptors resulting cells to enter in the cell cycle.

The aim of the research was to study the role of ERK 1/2 and JNK signaling pathways in the process of hepatocytes polyplodization in the cholestatic liver model.

Materials and methods. Experiments were carried out on adult white rats (130-150g). Model of cholestatic liver with common bile duct ligation was used. Animals of the first task were divided into three group: I-control intact animals, II –cholestatic animals (4th day), III-cholestatic animals with MEK 1/2 (PD98059) inhibitor injection (10mg/kg). Animals of the second task were divided into three group: I-control intact animals, II –cholestatic animals (4th day), III-cholestatic animals with JNK (SP 600125) inhibitor injection (1mg/kg).Nuclear DNA content was detected by using of computer software ImageJ 1.36 b. Determination of colchicine mitotic index was used for assessment of proliferative activity. Student's t test was used for comparison among the different groups. P<0.05 was considered statistically significant.

Results. The hepatocytes mitotic activity significantly increases on the 2nd day from common bile duct ligation in the first test group after inhibition of MEK 1/2. In addition, the number of diploid (2c) and binucleic (2c × 2) hepatocytes decreases and the polyploid (4c, 4c × 2, 8c) cells is increased. In the second test group of animals, the number of tetrapoloid cells (4c) has tendency to decreased, while octoploid (8c) and binuclear octoploid (4c × 2) cells are not present, which is evidence for suppression of polyploization. With regard to proliferative activity, there is no difference between the animals from first and second test group.

After the using of JNK inhibitor mitotic activity of hepatpcytes is increased in the first test group in compare to control in the 2nd day after the common bile duct ligation. At the same time the quantity of diploid (2c) and binucleic (2cx2) cells is decreased and polyploid cells (4c, 4cx2, 8c) is increased.

Increased number of polyploidy cells was seen also in liver of animals of the second test group, where no difference in mitotic activity between the first and second test groups was detected.

Conclusions. 1. Inhibition only of cell proliferation by the blocking of HGF and JNK cascade pathways in non-linear rats indicates that proliferation and polyploidization in the cholestatic liver is managed by different signaling pathways. 2. Inhibition of the formation of high-ploidy (octaploid) cells by blocking of MEK 1/2 proteins indicates that ERK 1/2 signaling pathway is one of thenecessary conditions for polyploidization of hepatocytes.