Секция «Тезисы на английском языке»

Editing the genome via CRISPR / cas9 in order to study the role of HHEX in the development of liver cirrhosis

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Hhex - a protein transcription factor which is involved in the regulation of blood cell development and differentiation of hepatocytes. It is shown that the level of its synthesis correlates inversely with the rate of cancer cell division, in addition, there are indications that it might be involved in the response to the toxic effects of hepatocytes. We hypothesized that Hhex may play a role in the development of fibrosis and cirrhosis.

Unfortunately, we do not have the genetic animal models to study the role Hhex in mature hepatocytes. Therefore, we decided to use the cell model by selecting as an object for study of human hepatocellular carcinoma cells of HepG2, which can be passaged indefinitely, and that, by virtue of origin, reminiscent of mature human hepatocytes.

There are two main approaches for studying the function of the protein in the cells - this overproduction or reduced levels of the protein in the cell in order to compare the properties of the modified cells with an altered level of protein and the original unmodified cells. Overproduction results are often difficult to interpret, so we decided to switch off the gene Hhex c in HepG2 cells using a genetic approach that uses CRISPR / Cas9 system for editing of the genome.

This system is based on the transient introduction into mammalian cells a genetic construct comprising cDNA Cas9 bacterial nucleases and short, so-called, gRNA (guide RNA), whose binding with the genomic DNA of complementarity principle defines the location of cutting nuclease Cas9. hus, in the right place to create a genomic DNA can be double-stranded break in a place which is inaccurate due to repair can be Daubal (or removed) several nucleotides. If the gap created in the early part of the gene coding can be achieved with high probability a frame shift that would lead to a functional protein knockout.

With the help of the program for the selection gRNA we picked up a short sequence of RNA to knock Hhex. A vector comprising the RNA, Cas9 gene and GFP fluorescent marker (to assess the proportion of transfected cells) were introduced into HepG2 cells using a flow cytometer sorted transfected cells. They were obtained as clones derived single cells and the total population of modified cells. The nature of the changes in the DNA isolated from individual clones by amplifying DNA fragments was determined by Sanger sequencing. As a result, we obtained HepG2 line with genetic knockout Nhex gene, which after further characterization will ispolzovny to study the role of this protein in the development of fibrosis (cirrhosis) of the liver.

Источники и литература

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