Effect of H2O2 on SIRT7 gene expression in Huh7

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Abstract

SIRT7 is a gene that controls protein synthesis. It is involved in the synthesis of several proteins and the formation of liver cancer.
H2O2 can affect the expression of genes in cells. In this research, H2O2 will be added into the Huh7, HA-SIRT cells and Huh7, HA- cells to see what its effect on SIRT7 gene expression in Huh7 is.

So in this experiment, the proteins of four different cells will be tested. They are Huh7, HA-SIRT with H2O2, Huh7, HA-SIRT without H2O2, Huh7, HA- with H2O2, and HA- without H2O2. By comparing the protein expression levels of these four cells, it can be inferred that the effect of H2O2 addition on SIRT7 gene expression in Huh7 cells. The tested proteins are SIRT7, Nrf2 R, and Lamin BL.

Western blot is an analytical technique widely used in molecular biology and immunogenetics to detect specific proteins in tissue homogenates or extracts. In a nutshell, protein denaturation is performed on the sample, followed by gel electrophoresis. A synthetic or animal-derived antibody (called a primary antibody) is created to recognize and bind to a specific target protein. The electrophoretic film is washed in a solution containing primary antibodies, and the excess antibodies are rinsed off. A secondary antibody is added, and the secondary antibody recognizes and binds to the primary antibody. Secondary antibodies can be visualized by staining, immunofluorescence, and radiation energy to detect specific target proteins indirectly. In this experiment, we focus on comparing the protein expression changes before and after adding H2O2 to the same cell. If there are any differences between them, that suggests that hydrogen peroxide does affect SIRT7. Because SIRT7 is involved in the synthesis of several proteins and the formation of liver cancer, this finding has the opportunity to interfere with SIRT7's role in cell carcinogenesis.

Introduction

The Huh7 cell is highly sensitive to the hepatitis C virus (HCV) and is often used as a model for liver cancer studies and the hepatitis C virus. The cell line can be used as an HCV replication subsystem, allowing in vitro production of infectious HCV particles and developing drugs against HCV (Sainz, TenCate & Uprichard, 2009).

SIRT7 is a gene that controls protein synthesis. SIRT7 is a member of the mammalian Sirtuins family, localized to the nucleolus, and is a highly specific deacetylase of H3K18Ac (acetylated lysine residue of histone H3). Recent studies have found that SIRT7 can participate in regulating ribosomal RNA transcription, cell metabolism, cell stress, DNA damage repair, and other physiological processes through a variety of pathways (Ford, Voit, Liszt, Magin, Grummt & Guarente, 2006). Besides, SIRT7 is also closely related to aging, heart disease, and fatty liver. In particular, SIRT7 plays an essential regulatory role in the occurrence and development of various tumors, such as liver cancer, stomach cancer, breast cancer, bladder cancer, colorectal cancer, and pancreatic cancer, and head and neck squamous cell carcinoma. In this research, edited DNA will be added into the Huh7 cells to see the SIRT7 expression and function. Huh7 cells added SIRT7 gene (called Huh7, HA-SIRT) and added SIRT7 knockout gene (called Huh7, HA-) would be used. The HA- is a precursor to connecting the SIRT7 gene as a way to check if the packet virus is successful.

H2O2 can affect the expression of genes in cells. After a certain number of Huh7 cells have been cultured in this project, H2O2 will be added. About one hour later, the proteins from the cells will be immediately taken out for quantitative testing. Western blotting will be used to measure the concentration of different proteins in the cell. To conduct a controlled experiment, the protein content of another group of cells without H2O2 will also be measured.

Materials and Methods

Results and Discussion

According to the experimental results, H2O2 did affect the effect of SIRT7.
However, due to the limitation of the length of the experiment, I only did three complete experiments, and the results of each experiment were not similar to each other, so I could not draw an effective conclusion.

Reference

Ford, E., Voit, R., Liszt, G., Magin, C., Grummt, I., & Guarente, L. (2006). Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. Genes & development, 20(9), 1075-1080.
Sainz, B., TenCate, V., & Uprichard, S. L. (2009). Three-dimensional Huh7 cell culture system for the study of Hepatitis C virus infection. Virology journal, 6(1), 1-8.