JCOMS eISSN:2783-3127 https://journalofcoms.com

Journal of

Current Oncology and Medical Sciences

Vol. 3, No.3

Original Free Access

The effect of consumption of Agaricus blazei edible mushroom on caspase2 gene expression in hepatitis C patients using system biology and microarray data

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Abstract

Introduction: Agaricus blazei mushroom is used as a food and medicine, its effective composition is beta-glucan, which is used to treat some cancers and infections, including hepatitis C.Hepatitis C is an inflammatory disease that causes liver necrosis. Caspase2 protein is one of the factors promoting cell apoptosis and plays a role in tumor suppression. The purpose of this study is to determine the expression changes of the caspase2 gene and its effects on liver cancer.

Materials and Methods: In this project, raw expression data was obtained from the NCBI (National Center for Biotechnology Information) GEO (Gene Expression Omnibus) database section and using bioinformatics tools and methods and system biology such as Matlab (An abbreviation of "MATrix LABoratory), GEOR2 (Online software) and Cytoscape, the effect of consuming the desired mushroom on caspase2 gene expression was investigated.

Results: It was found that the beta-glucan combination has an increasing effect on target gene expression (p-value=0.05692).

Conclusion: The results show that the beta-glucan present in the mushroom can play a role as a prevention and even treatment of liver cancer by increasing the expression of caspase 2 protein by directing the damaged cell towards apoptosis.

Keywords: Beta-glucan, Agaricus blazei, hepatitis C, caspase2, Microarray, System biology

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Received: 2023.5.16, Accepted: 2023.9.23

Introduction

Agaricus blazei belongs to the category of basidiomycetes or umbrella mushrooms, which is one of the food items on people's tables in most countries. This mushroom is mostly native to Brazil and Japan and is currently used as one of the edible and medicinal mushrooms that are traditionally used to treat many common diseases such as arteriosclerosis, hepatitis, blood lipids, diabetes, and cancer. This mushroom also has immunomodulatory and anti-mutagenic properties (1). One of the most important effective compounds in edible mushrooms Agaricus blazei is a beta-glucan polysaccharide (2,3). This type of mushroom and even different types of edible mushrooms are used to treat some cancers and infections, including hepatitis C, due to the presence of beta-glucan polysaccharide (4). Hepatitis C is a type of contagious infection that mainly affects the liver and is caused by a virus that attacks the liver and causes inflammation of the liver (5). Most people infected with the hepatitis C virus do not feel any symptoms, in fact, many of them may not even know that this virus is present in their body (6). Hepatitis C can be chronic or acute. Acute hepatitis C is a short-term illness that occurs within the first 6 months of being infected with this virus and lasts for a few weeks. In most people, acute hepatitis C turns into chronic hepatitis C. Chronic hepatitis C is a long-term disease and occurs when the hepatitis C virus remains in the body for a long time (7). Hepatitis C virus infection can cause serious liver problems such as liver cirrhosis or liver cancer (8). Caspase 2 protein is one of the tumor suppressors. Caspase 2 may be an important protector against tumorigenesis (9). A hallmark of tumorigenesis is that cancer cells avoid apoptosis, so caspase 2 may suppress tumors by inducing apoptosis of potential tumor cells. Alternatively, caspase-2 protein may suppress tumor growth by inducing cell cycle arrest (10). In addition, it has been observed that caspase 2 causes cell cycle arrest after DNA (Deoxyribonucleic acid) damage (11). Microarray is one of the most widely used methods of generating Big data related to gene expression levels in genome function projects (12). One of the best storage databases is the GEO gene expression data set, which is located in the NCBI. In this research, to investigate the increasing or decreasing effect of beta-glucan consumption on caspase 2 gene expression, the gene expression data of the project with accession number Gse3983, which is available in the NCBI database as raw data, was used. Therefore, in order to find out the effect of beta-glucan on increasing or decreasing the expression of the studied gene, the raw data of the microarray has been measured and analyzed with bioinformatics software.

Materials and Methods

To get the data related to the expression of the caspase2 gene, refer to the research carried out under the name of Gse3983, which was conducted on four patients. These patients included three male and one female. The patients (3 males one female, 48-56 years) had chronic hepatitis C infections that did not respond to interferon/ribavirin treatment. They were given one week of treatment with Agaricus extract (Gold Label from ACE Co, Japan, 20 ml 3x daily). Blood samples were drawn before and after treatment. In order to investigate the effect, we examined the changes of gene expression caused by the extract on a human monocyte cell line (THP-1). Changes in the levels of mRNA transcripts were measured using 35k microarrays (13). Microarray experiments provide the scientific community with huge amounts of data. Without appropriate methodologies and tools, significant information and knowledge hidden in these data may not be discovered. Therefore, there is a need for methods capable of handling and exploring big data sets. The field of data mining and machine learning provides a wealth of methodologies and tools for analyzing large data sets (14). The raw data of this research has been presented in NCBI. The raw data of the caspase2 gene has been published among thousands of genes that were the result of microarray operations. By referring to the NCBI website and after receiving the raw data from the GEO data sets section for pre-processing the data, analysis and to determine the desired gene expression, MATLAB software was used, and Cytoscape software were used to show the relationships of genes in terms of genetic relationships with other genes. Also, GEO2R has been used to analyze the data and examine the changes in the desired gene expression in two groups of patients, i.e., a group of four patients before and after treatment. Matlab is a programming and numeric computing platform used by millions of engineers and scientists to analyze data,

develop algorithms, and create models. Using toolbox functions of matlab, we can read genomic and proteomic data from standard file formats such as SAM, FASTA, CEL, and CDF, as well as from online databases such as the NCBI Gene Expression Omnibus and GenBank. Cytoscape is an open-source software project for integrating biomolecular interaction networks with high-throughput expression data and other molecular states into a unified conceptual framework. GEO2R is an interactive web tool that allows users to compare two or more groups of Samples in a GEO Series in order to identify genes that are differentially expressed across experimental conditions. In this study, MATLAB R2018A and Sytoscape 3.10.0 were used.

Results

By executing the command codes in the MatlabR2018a software, the clustergram of figure 1 was obtained.

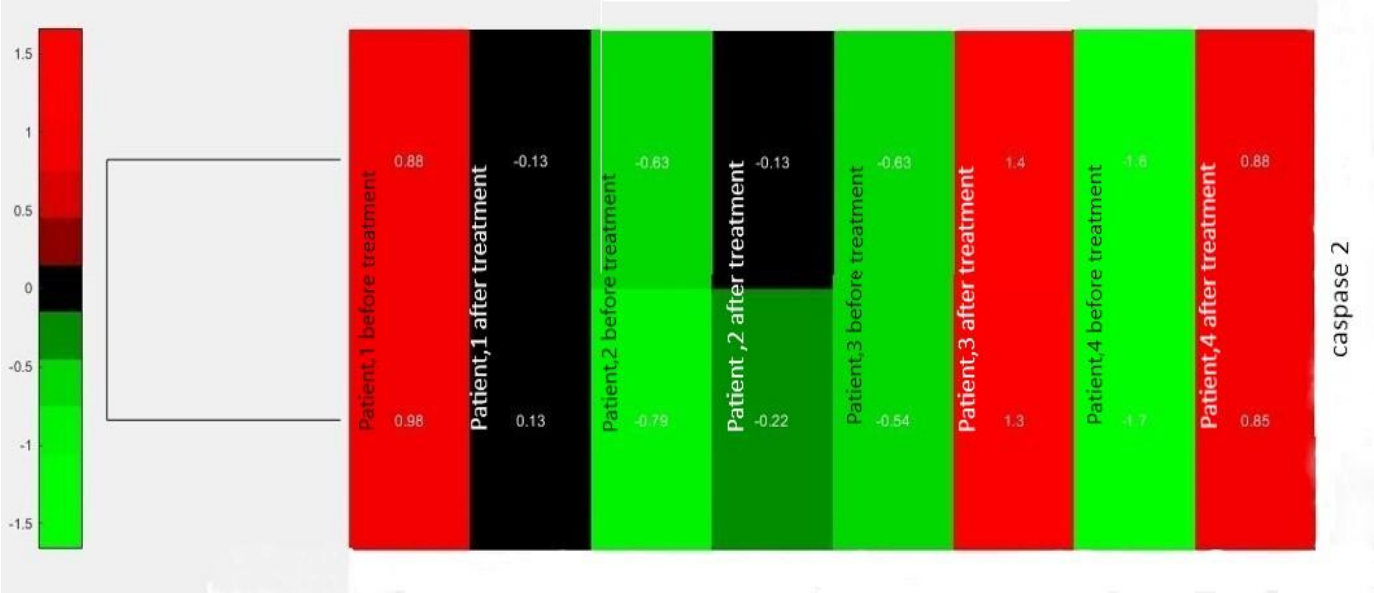

Figure 1. Caspase2 gene expression clustergram: In this clustergram, the sample before and after beta-glucan intake is written in the horizontal line, and the gene name is inserted in the upper line. Light red color indicates the highest and light green color indicates the lowest expression. This clustergram shows the increase or decrease of caspase 2 gene expression in all four patients before and after treatment with beta-glucan. As can be seen in figure, based on the analysis of microarray data, in the first patient after treatment with beta-glucan, the expression of caspase 2 gene has decreased, but in the other three patients, treatment with beta-glucan has increased the expression of caspase 2 gene.

In this clustergram, the horizontal line indicating each individual (sample) before and after beta-glucan consumption and the gene name is inserted in the vertical line are specified. Light red color indicates the highest expression and light green color indicates the lowest expression. Based on this, the results of the clustergram study Differences in the relative expression of the caspase 2 gene were detected as increases. Also, another method called GEOR2 was used, which showed the previous result, that is, we saw a significant (Pvalue = 0.05) increase in expression. The result of increased expression by GEOR2 is shown in figure 2. The genetic relationship of the caspase2 gene with other genes is shown in figure 3.

ID	P-VALUE	Gene.symbol	Gene.title
211028	0.05692	CASP2	caspase 2

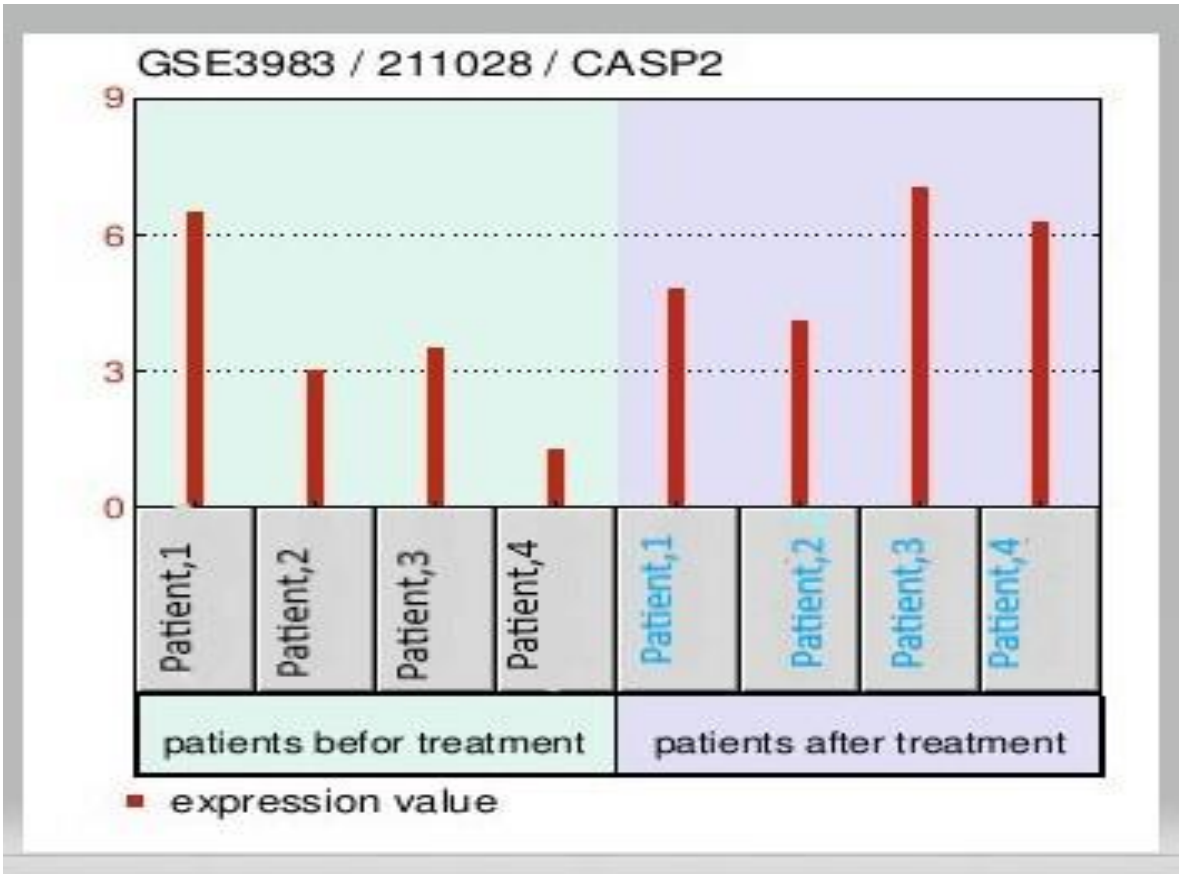

Figure 2. Caspase 2 gene expression graph from GEOR2 method: In these graphs, the expression of this gene is shown in all four patients before and after the consumption of Agaricus blasi mushroom beta-glucan .As can be seen in figure 2, based on the analysis of microarray data, in the first patient after treatment with beta-glucan, the expression of caspase 2 gene has decreased, but in the other three patients, treatment with beta-glucan has increased the expression of caspase 2 gene.

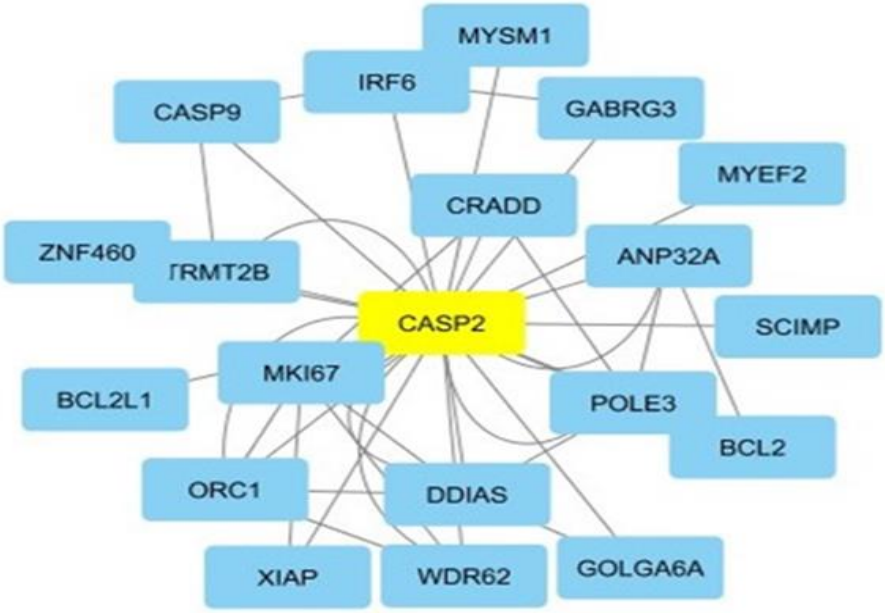

Figure 3. The genetic connection of caspase 2 gene with other genes: The genetic connection of caspase 2 gene with other genes is shown in the form of communication bridges.

Discussion

Beta-glucan is one of the natural polysaccharides found as a component of the cell wall in grains, algae or in some microorganisms such as bacteria, fungi and yeasts, and by examining beta-glucan in the 1960s and 1970s, researchers well established the important role of beta-glucan in The immune system has been proven to treat cancer, infection, and repair damaged bone marrow (4). Hepatitis C is an inflammatory liver necrosis disease that occurs acutely and chronically. The causative agent of the disease is a virus from the flavivirus family and has a high tendency to reproduce in liver tissue (7). Currently, about 170 million people in the world have chronic hepatitis (8). 7 to 20% of these patients suffer from liver cirrhosis (7). On the other hand, cirrhosis causes serious complications such as liver cancer (15). Today, it is known that caspase 2 is the most important member of apoptosis and the coordinator of the death pathway or the activation of other caspases (9). And the defect in the process of apoptosis is the cause of many diseases including cancer (16). Caspase 2 is one of the tumor suppressors and recent studies show that caspase 2 may play a role in suppressing tumorigenesis (9). Caspase 2 protein expression strongly localizes to injured/ballooned hepatocytes (17). Caspases mediate cell apoptosis after stimulation; they can be divided into initiators of apoptosis, such as caspase 2, 8, 9, 10, and 12, and effectors of apoptosis, such as caspase-3, 6, and 7 (18). the present study, it was shown that beta-glucan present in edible mushroom Agaricus balzei increased the expression of caspase 2 protein. Therefore, caspase 2 may suppress tumors by inducing apoptosis of potential tumor cells (10). Considering that caspases play an essential role during cell death and changes in caspases are involved in the development of human cancer (19). on the other hand, in a study conducted on gastric cancer by immunohistochemistry using a tissue microarray approach, it was determined that the expression of Caspase 2 gene has changed and these changes are reduced in gastric cancer cells compared to normal cells (17). Therefore, it can be said that the beta-glucan present in the edible mushroom Agaricus blazei can increase the expression of caspase-2 protein and thereby lead the damaged liver cells in hepatitis C patients to apoptosis. In the end, it is suggested that the effect of beta-gluganan be done in a laboratory and real-time PCR method on patients with hepatitis C disease and the results be compared with the results of the research.

Conclusions

Therefore, according to the materials and sources mentioned in the discussion section, as well as using the results of microarray data analysis, it seems that beta-glucan in the edible mushroom Agaricus blazei can act as a preventive by guiding the damaged liver cells in hepatitis C patients by increasing the expression of caspase 2 protein. and even treat liver cancer.

Author contribution

All authors have equal contribution.

Conflict of interest

The authors have no conflict of interest to declare.

Funding

There is no funding.

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