

The Effect of Allicin on ZNF703 Gene Expression in GCC Lines

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Abstract: *Garlic (Allium sativum), a member of the family Liliaceae, contains an abundance of chemical compounds that have been shown to possess beneficial effects to protect against several diseases, including cancer. Evidence supports the protective effects of garlic in stomach, colorectal, breast cancer in humans. The exact mechanisms of the cancer-preventive effects are not clear, although several hypotheses have been proposed. Oil-soluble compounds derived from garlic, such as diallyl disulfide (DADS), are more effective than water-soluble compounds in suppressing breast cancer. MTT assay and flow cytometry were used for the evaluation of the effect of allicin on gene expression. The reverse transcription polymerase chain reaction (RT-PCR) and western blot analysis were respectively used for the analysis of ZNF703 gene expression. The results revealed that exposure of the gene to allicin at a concentration of 40 μ M significantly promoted the cell viability. Treatment of the cells with 10, 20, 30, 40, and 50 μ g/mL of allicin enhanced the cell viability by $2.5.47 \pm 0.86$, 5.43 ± 0.66 , 10.74 ± 1.48 , 35.89 ± 3.78 , and $32.21 \pm 2.92\%$, respectively after 36 h compared to control cells. Allicin exposure caused a marked decrease in the percentage of cells in G0/G1 phase with a subsequent increase in the S phase population.*

Keywords: *Allicin, ZNF703, Gene Expression, GCC Line.*

1. Introduction

The name “*Allium sativum*” is derived from the Celtic word “all”, meaning burning or stinging, and the Latin “*sativum*” meaning planted or cultivated. The English word, garlic, is derived from the Anglo-Saxon “gar-leac” or spear plant, referring to its flowering stalk. Allicin is a strong antibiotic has a long traditional medicinal importance and was used to treat a range of diseases. The major compound present in the extract of garlic is the allicin which on rearrangement forms ajoene. It is reported that the organo-sulfur compounds like S-allylcysteine present in the garlic exhibit inhibitory effect on the Gene Expression in GCC Lines [1].

The zinc finger protein 703 (also known as ZPO1, ZEPP01) gene is located on chromosome arm 8p12. The ZNF703 protein belongs to the NET (Noc/Nlz, Elbow, and Tlp-1) family, which plays an important role in the embryonic development of zebrafish [2] and Drosophila [3]. It has been shown that ZNF703 gene amplification stimulates migration and proliferation while reducing cell to cell adhesion [4]-[6] and is speculated to be associated with poorer outcomes [5]-[8] in breast cancer. Two studies from China also discovered that ZNF703 acts as an oncogene that accelerates malignant progression in gastric cancer [9] and is associated with worse prognosis in colorectal cancer patients. [10].

Guanylate cyclase 2C, also known as guanylyl cyclase C (GC-C), intestinal guanylate cyclase, guanylate cyclase-C receptor, or the heat-stable enterotoxin receptor (hSTAR) is an enzyme that in humans is encoded by

the *GUCY2C* gene. [11], [12] Guanylyl cyclase is an enzyme found in the luminal aspect of intestinal epithelium and dopamine neurons in the brain. [13] The receptor has an extracellular ligand-binding domain, a single transmembrane region, a region with sequence similar to that of protein kinases, and a C-terminal guanylate cyclase domain. Tyrosine kinase activity mediates the GC-C signaling pathway within the cell.

Garlic has historically been used to treat earaches, leprosy, deafness, severe diarrhea, constipation and parasitic infections, and to lower fever, fight infections and relieve stomach aches. Garlic and its extracts have been used to treat infections for thousands of years [14] and it has long been revered for its medicinal properties as evidenced by ancient writings from Egypt, Greece, China and India extolling its merits. Garlic is thought to have diaphoretic, expectorant, antispasmodic, antiseptic, bacteriostatic, antiviral, antihelminthic and hypotensive effects; it is commonly used to treat chronic bronchitis, recurrent upper respiratory tract infections and influenza. [15] It has been used for medicinal purpose for more than 3000 years, and has bactericidal, [16] antibiotic, [17] and fungicidal [18] properties. Epidemiologic and preclinical studies suggested that garlic may influence the risk of heart disease and cancer [19] and also as an anticancer dietary component are reported by Fleischauer and Arab. [20] The most compelling evidence that garlic and related sulfur constituents can suppress cancer risk and alter the biological behaviour of tumors. Experimentally, garlic and its associated sulfur components are reported to suppress tumor incidence in breast, colon, skin, uterine, esophagus and lung cancers. [21] A recent meta-analysis also showed that a high intake of garlic may be associated with decreased risks for breast cancer. Garlic has historically been used to treat earaches, leprosy, deafness, severe diarrhea, constipation and parasitic infections, and to lower fever, fight infections and relieve stomach aches.

2. Material and Method

Alliin was isolated from the extract of *Allium sativum* (garlic, lasun) using traditional column chromatography. The stalk solution of alliin was prepared in dimethyl sulphoxide and stored at -20°C prior to use. MTT assay and flow cytometry were used for the evaluation of the effect of alliin on gene expression. The reverse transcription polymerase chain reaction (RT-PCR) and western blot analysis were respectively used for the analysis of ZNF703 gene expression.

3. Results

The results from RT-PCR and western blot analysis revealed a marked enhancement in ZNF703 expression in the GCC line on treatment with alliin for 36 h ($P < 0.05$). The expression levels of the gene corresponding was also increased ($P < 0.05$).

4. Discussion

Several pieces of evidences suggested that *Alliin* possess anticancer properties as shown by their ability to suppress tumor proliferation in vivo and in vitro. [22] It was shown that concentration and duration of the exposure to allyl sulfides increased the antiproliferative effects. In our study the amount of Alliin formed from *A. hirtifolium* was $3.4 \pm 0.1 \text{ mg g}^{-1}$ and the theoretical amount of Alliin formed from garlic is reported as 3.5 mg g^{-1} . [23].

This antineoplastic effect was greater for lipid-soluble than for water-soluble allyl sulfides. [24] Diallyl disulfide suppresses the growth of human colon tumor cell xenografts in athymic nude mice. [25].

Allium sativum (garlic) and other onions. This species is a native plant in Iran that its antiproliferative effects have received little attention. [26] In the present study we assessed the antiproliferative effect of *Alliin* extract on ZNF703, and GCC cell lines by MTT method and the DNA fragmentation analysis. This investigation clearly showed a cell growth inhibition on cancer cell lines at concentrations about $44 \mu\text{g ml}^{-1}$ of *Alliin* extract. The effect of *Alliin* on these cells started from 24 h and became more prominent in 48 and 72 h. Therefore, maximum morphologic changes and antiproliferative effect appeared after 72 h. In MTT assay, the statistical analysis indicated that *Alliin* extract significantly inhibited the proliferation of ZNF703 cells. Interestingly the *Alliin* extract affected the tumor cells much strong.

The results showed that *Allicin* could inhibit proliferation of tumor cell lines in a dose-dependent manner. The inhibitory effect on tumor cell lines was 25 times stronger than that in normal cells. Therefore, our results provided important insights into the use of *Allicin* as an additive to food or as a drug without any side effects.

The present study was the first report to provide evidence of *Allicin* activity on tumor cell lines. Further investigations were needed to elucidate subcellular mechanisms involved in the suppression of growth in tumor cell lines.

5. Conclusion

The results of this study showed that allacin enhances ZNF703 gene expression in GCC line.

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