

The effects of Alicine on ZNF703 Gene Expression in Gastric Carcinoma Cell Line

Naji T, Hatami F*, Ganjipour G, and Kheirvari Khezerloo J

Abstract—Cancer is the genetic disease of the somatic cells, which defects in normal cell division or the failure of the programmed cell death pathway is its major cause. Although we have witnessed major developments in the medical science during the past decades, the cure for cancer is still the biggest challenge of the medicine. Garlic (*Allium sativum*) contains Allicin, which is an active chemical with antioxidant and anticancer properties. In this study, ZNF703 gene reported as a factor involved in stomach cancer, the lethal effect of garlic extract on AGS cells were studied in a period of 24 h with MTT test. Data regarding different concentrations of extract were gathered by an ELISA reader and IC50 was calculated. Subsequently, AGS cells were treated with 780 µg of extract. After 24h, RNA extraction and cDNA synthesis was done and ZNF703 expression was analyzed by Real-Time PCR. Our results indicate that ZNF703 expression changes with different concentrations of garlic extract and has an inhibitory effect on ZNF703 expression. In conclusion it is suggested that research aimed at characterization of anti-cancer effects of garlic extract is indeed promising.

Index Terms— Garlic, Stomach Cancer, ZNF703 genes, AGS Cell Line.

I. INTRODUCTION

Allicin, a chemical found in garlic, crushed or chewed there. Allylsine is a derivative of lysine, used in the production of elastin and collagen. It is produced by the actions of the enzyme lysyl oxidase in the extracellular matrix and is essential in the crosslink formation that stabilizes collagen and elastin. Allicin is very powerful as an antibiotic that inhibits the ability to grow and multiply and helps microbes anti-viral, fungal and bacterial. ZNF703 [1] is a gene which has been linked with the development of breast cancers. ZNF703 is one of the family members of yellow NET/NLP and a zinc finger transcription factors. ZNF703 is a part of 8p12 telomeric amplicon that is associated with Luminal B breast cancer. ZNF703 function in luminal B breast cancer Transcription of the amplified ZNF703 gene is activated by E2-ESR1 signalling. ZNF703 forms a nuclear repressor complex together with DCAF7, HSP60 and PHB2 (Prohibitin 2) [1]-[3]. ZNF703 was found to co-localize with HDAC1 on

promoters with H3Kme1 or H3K4me3 labels suggesting mediation of active repressing of genes such as PAX2 and TGFβP2 [4]-[6]. Stomach cancer, also known as gastric cancer that developing from the lining of the stomach. The most common cause is infection by the bacterium *Helicobacter pylori*, which accounts for more than 60% of cases. Early symptoms may include heartburn, upper abdominal pain, nausea and loss of appetite. Later signs and symptoms may include weight loss, yellowing of the skin and whites of the eyes, vomiting, difficulty swallowing, and blood in the stool among others [7]. The cancer may spread from the stomach to other parts of the body, particularly the liver, lungs, bones, lining of the abdomen and lymph nodes [8]. As has been proven Medicinal herbs and their derivative phytochemicals are being increasingly recognized as useful complementary treatments for cancer [9]. A large volume of clinical studies have reported the beneficial effects of herbal medicines on the survival, immune modulation, and quality of life (QOL) of cancer patients, when these herbal medicines are used in combination with conventional therapeutics [10]. Research shows that green tea consumption may reduce the risk of cancers of the colon, rectum and pancreas is examined in a large population-based case-control study conducted in Shanghai, China [11] And also the effectiveness of Chinese medicinal herbs in the short term remission of advanced or late gastric cancer have been proved. In another study the scientists found out the effect of Paeonol inhibition of tumor growth in gastric cancer in vitro and in vivo [12]. Studies Also show that Ginger ingredients reduce viability of gastric cancer cells via distinct mechanisms [13]. Effect of rikkunshito, a Japanese herbal medicine, on gastrointestinal symptoms and ghrelin levels in gastric cancer patients after gastrectomy [14] Diallyl sulfide, a flavor component of garlic (*Allium sativum*), inhibits dimethylhydrazine-induced colon cancer [15]. Recently published results of epidemiologic case-control studies in China and Italy on gastric carcinoma in relation to diet suggest that consuming garlic may reduce the risk of gastric cancer, Epidemiological studies reveal an inverse relationship between garlic consumption and death rate for gastric cancer in populations in China. These reports suggest a role for garlic in the prevention of human cancer [16]. Chemical constituents of garlic have been tested for their inhibiting effect on carcinogenesis, using in vitro and in vivo models. In most experiments inhibition of tumour growth was established using fresh garlic extract, garlic compounds or synthetically prepared analogs. [17]. Another study shows the Effects of *Helicobacter pylori*, Garlic, and Vitamin Treatments on Gastric Cancer Incidence and Mortality [18]. A Research results demonstrate Comparison of combined effects of 5-fluorouracil and allysin after concurrent treatment in colon and breast cancer cell lines [19]. Researchers shows that the extract of garlic oil has the ability to adjust intra

Tahereh Naji (PhD) is with the Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS).

Forough Hatami (*corresponding author) is with Department of Molecular and Cellular Science, Faculty of Biology Science, Tehran North Branch, Islamic Azad University, Tehran, Iran.

Ghazaleh Ganjipour is with Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS).

Jamil Kheirvari Khezerloo is with Department of Biochemistry, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS).

cellular genes specially tumor suppressor genes [20]. Also researchers found that the expression level of ZNF703 protein is higher than normal level in Gastric mucosa in Stomach cancer tissue [21]. There is also a research that indicated the effect of Garlic consumption on cancer prevention meta-analyses of colorectal and stomach cancers shows that the expression of ZNF70314 Decrease [22]. Cancer is the genetic disease of the somatic cells, which defects in normal cell division or the failure of the programmed cell death pathway is its major cause. Although we have witnessed major developments in the medical science during the past decades, cure for cancer is still the biggest challenge of the medicine as its becoming prevalence and also Causing clinical complications and high costs for patients. According to the limitation of studies in this research area, the present study carried out and investigates effect of alicine on Znf703 gene expression in gastric carcinoma cell lines. The results of this study will be important in understanding gastric cancer treatment.

II. MATERIAL AND METHODS

In This laboratory-experimental study AGs cell lines RPMI cell cultures, penicillium, Fetal bovine serum, Paints for MTT test and also Alcoholic extract of garlic were used which have been prepared by Islamic Azad University of Zanjan branch. Garlic was prepared in different concentration (1.20, 1.40, 1.80, 1.160, 1/320, 1/640 mg/ml) as were prepared in Previous studies. Gastric adenocarcinoma cell line was purchased from the cell bank of Iran Pasteur Institute. RPMI medium was used to Cultivate the tumor cells. To prepare the phosphate buffered saline PBS tablets were purchased from Sigma Company. RPMI medium was used to Cultivate the tumor cells. RPMI 1640 medium containing sterile liquid Heps / Celine antibiotic penicillin / streptomycin / Trypsin-solution (EDTA 1X) biosera company was purchased as a ready. RPMI medium was used as a medium to prepare a culture medium to medium Celine antibiotic penicillin / streptomycin at a final concentration of 100 units per ml and 100 mg/ml, respectively, and then added to a concentration of fetal bovine serum the final 10% was added to the medium. After replacing the culture medium the cells were passaged divides them based on speed, In order to better growth of cells was performed every 3 days changing medium. The cells were frozen and then thawed. To investigate the effect of garlic extract for cell viability MTT assay method was used in this study. We used produced cDNA in real time-PCR after the reverse transcription. The quantity and quality of extracted RAN obtained with nanodrop and agarose gel electrophoresis. In this study, tag man probe was used for the the ZNF703 and beta-actin gene expression using Real-time PCR. The lyophilized primers and probes were synthesized by Pioneer Company. All experiments were performed twice, average comparison by using Graph pad prim software and using SPSS 16 software and ANOVA, post hoc Turkey test and correlation analysis was performed on data And to determine the significance of differences was performed at the level of $p < 0.05$.

III. RESULTS

Figure 1 shows the viability of AGs cells in response to different doses of garlic extract in 24 hours. AGs cells treated with various concentrations of garlic extract at concentrations

of 100, 400, 800 and 1000 mg/ml within 24 hours have significant change compare to control group. In order to reduce cell viability by MTT assay. On the results of the IC 50 garlic extract on the AGs cells to 780 mg/ml. As specified in the form of a concentration of 1000 mg of garlic extract at a concentration of 100 mg lowest and highest inhibitory effect on cell growth inhibition effect of AGs.

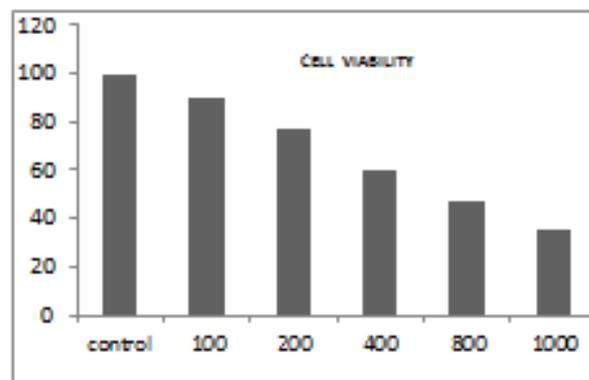


Fig. 1. AGs cells viability in response to different doses (mg/ml) of garlic extract in 24 hours

IV. DISCUSSION

Garlic (*Allium sativum*) contains Allicin, which is an active chemical with antioxidant and anticancer properties. In this study, ZNF703 gene reported as a factor involved in stomach cancer, the lethal effect of garlic extract on AGS cells were studied. The results of this study suggest garlic in the concentration of 1000 mg Dadsarh most lethal effect at a concentration of 100 micrograms least had the effect of property. Based on the results obtained from the IC 50 is 780 mg of garlic extract for the cell. According to the study garlic extract in high dose has been reduced cell growth AGS. It has also been observed in previous studies and recent, researchers found that the expression level of ZNF703 protein is higher than normal level in Gastric mucosa in Stomach cancer tissue. also a research that shows the effect of Garlic consumption on cancer prevention meta-analyses of colorectal and stomach cancers shows that the expression of ZNF70314 Decrease [21-22] Our results indicate that ZNF703 expression changes with different concentrations of garlic extract and in fact, the extract has an inhibitory effect on ZNF703 expression.

V. CONCLUSION

We have shown that high dose of garlic extract has anticarcinogenic effect on AGS cells in vitro also it is suggested that research aimed at characterization of anti-cancer effects of garlic extract is indeed promising.

ACKNOWLEDGMENT

We appreciate all who helped us to exert the present study. This paper reports a part of results selected from research work carried out by Ghazaleh Ganjipour supervised by Jalil Fallah Mehrabadi and Consulted by Dr. T. Naji at Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS).

REFERENCES

- [1] Serge ankri, David mirelman. Review: antimicrobial properties of allicin from garlic. 10/2016

- [2] Tsan-Chang ChangHung-Der JangPeng-Fu Duan, Antioxidant and antimicrobial activities of commercial rice wine extracts of Taiwanese *Allium fistulosum*. January 2016
- [3] peter josling.allysin the heart of garlic.book
- [4] Bazarov,A.Yasawen,P.2011,who is in the drivers seat in 8p12 amplifications?ZNF703 in luminal B breast tumors,breast cancer res,vil.13,p.308.
- [5] Ginestier,C.[et Al] 2011,ZNF703: a novel oncogene involved in breaat cancer,Medicine science
- [6] Zi zhang[et al]ZNF703 gene amplification at 8p12 specifies luminal B breast cancer,Aug2013
- [7] malakzade,R.gastric cancer in Iran.2009
- [8] Kazuhiro ishiguro.Ginger ingredients reduce viability of gastric cancer cells via distinct mechanisms.oct2008
- [9] Tao gun,[et al].Chinese herbal medicines for induction of remission in advanced or late gastric cancer.jan2010
- [10] Therapeutic Applications of Herbal Medicines for Cancer Patients.Shu-Yi Yin, Wen-Chi Wei, Feng-Yin Jian, and Ning-Sun Yang jul2013
- [11] Induction of Apoptosis by *Saussurea lappa* and *Pharbitis nil* on AGS Gastric Cancer Cells.Seong-Gyu Ko1) 2), Seung-Hee Koh3), Chan-Yong Jun3), Chang-Gyu Nam4), Hyun-Su Bae5), Min-Kyu Shin5).2004
- [12] Małgorzata Milczarek, Anna Szelągowska, Katarzyna Lubelska, Marcin Bieńko, Dariusz Matosiuk, Zdzisław Chilmoneczyk, Katarzyna WiktorskaThe combined effect of 5-fluorouracil and sulforaphane in prostate cancer cell line.
- [13] Ginger ingredients reduce viability of gastric cancer cells via distinct mechanisms, October 2007, Kazuhiro Ishiguroa, , Takafumi Andob, Osamu Maedab, Naoki Ohmiyab, Yasumasa Niwab, Kenji Kadomatsuc, Hidemi Gotob.
- [14] Shuji Takiguchi, Yuichiro Hiura, Tsuyoshi Takahashi, Yukinori Kurokawa, Makoto Yamasaki, Kiyokazu Nakajima, Hiroshi Miyata, Masaki Mori, Hiroshi Hosoda, Kenji Kangawa, Yuichiro Doki. Effect of rikkunshito, a Japanese herbal medicine, on gastrointestinal symptoms and ghrelin levels in gastric cancer patients after gastrectomy. April 2013, Volume 16, Issue 2, pp 167–174.
- [15] Diallyl sulfide, a flavor component of garlic (*Allium sativum*), inhibits dimethylhydrazine-induced colon cancer
- [16] *Allium sativum* (Garlic) and cancer prevention, Benjamin, Lau, Padma P. Tadi, Jeffrey M. Tosk. 10/2016.
- [17] Garlic and its significance for the prevention of cancer in humans: a critical view. Dorant E1, van den Brandt PA, Goldbohm RA, Hermus RJ, Sturmans F.
- [18] Fifteen-year effects of *Helicobacter pylori*, garlic, and vitamin treatments on gastric cancer incidence and mortality. Ma JL1, Zhang L, Brown LM, Li JY, Shen L, Pan KF, Liu WD, Hu Y, Han ZX, Crystal-Mansour S, Pee D, Blot WJ, Fraumeni JF Jr, You WC, Gail MH.jan2012
- [19] Fluorouracil response in a large panel of colorectal cancer cell lines is associated with mismatch repair deficiency,K Bracht,I A M Nicholls,I Y Liu,1,2 and W F Bodmer1,*jul2010
- [20] Effects of garlic oil on tumorigenicity and intercellular communication in human gastric cancer cell line.Li X1, Xie J, Li W, Ji J, Cui J, Zhao M, Sun M, Lü Y.
- [21] metastase and grows of tumours by expression of znf703.Reynisdottir [et al],2013
- [22] smaldone and davice. 2010.