ORIGINAL ARTICLE

Received: June 2018
Accepted: September 2018

Evaluating Gelsolin Gene Expression Among Iranian Breast Cancer Patients

Sara Tutunchi[1], Mojtaba Saffari[2], Mahta Mazaheri[1], Parisa Nourmohammadi[1], Fereidoon Memari[3], Reza Shirkoohi[4,*], Nasrin Ghasemi[5,*]

1. Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran. I.R Iran.
3. Iran National Tumor Bank, Cancer Research Center, Cancer Institute of Iran, Tehran University of Medical Sciences, Tehran, Iran.
4. Cancer Biology Research Center, Cancer Institute of Iran, Tehran University of Medical Science, Tehran, Iran.
5. Recurrent Abortion Research Centre, Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

***Corresponding Authors:**

Reza Shirkoohi[#,1]

Nasrin Ghasemi[#,2]

Imam Khomeini Hospital Complexes, Cancer Institute, Cancer Research Center, Department of Genetics, Section of Molecular Genetics, Tehran, Iran.

Tel: (+98)21 66914545

Fax: (+98)21 66581638

Email 1: rshirkoohi@tums.ac.ir

Email 2: nghasemi479@gmail.com

BCCR

2018; 10(4): 16-25

www.bccrjournal.com

ABSTRACT

Background: Breast cancer is the most common type of cancer among women, and is among the five most prevalent cancer types among Iranian women. Advanced-stage breast cancer is often correlated with distant metastases. Alterations in gene expression affect general cytoskeleton changes during differentiation and oncogenesis, and can be considered an important factor in tumor progression. Gelsolin plays a significant role in actin assembly and has been introduced as a tumor activator. The aim of this study was to evaluate the expression of Gelsolin in breast cancer as well as its correlation with patients' clinical parameters.

Methods: In this study, 70 breast cancer patients, who had been referred to Cancer Institute, Imam Khomeini Hospital Complex for surgery were randomly selected. Normal and tumor tissues were prepared and stored at -80°C. Gelsolin gene expression was measured using real-time polymerase chain reaction (PCR).

Results: The results showed that Gelsolin gene expression had increased in 68.6% of the tumor samples. In addition, there was a significant association between increased levels of gene expression and tumor progression stages ($P < 0.05$). However, there was no significant association between increased levels of gene expression and other clinical findings, such as tumor grade, tumor size or patient age.

Conclusion: The results revealed that Gelsolin gene expression had increased in the tumor samples. Gelsolin overexpression also resulted in increased lymph node involvement in breast cancer. The expression of this gene also increased significantly during advanced stages of breast cancer; however, there was no significant relation between Gelsolin expression and tumor grade or tumor size.

Keywords: **breast cancer, gelsolin expression, Iranian population, tumor grade, tumor size**

INTRODUCTION:

Despite considerable medical progresses, cancer remains one of the most important health issues of the day, and is the second cause of death worldwide, particularly in developed and developing countries[1]. By 2020, it has been predicted that new incidents of cancer will be diagnosed in 15 million patients, and 60% of these will occur in developed countries[2,3]. Breast cancer is among the top five most prevalent cancer types in Iran and is the most common cancer among Iranian women[4]. Breast cancer develops via the progressive branching system of ducts that originate at the nipple and end in one of many terminal ductal lobular units (TDLUs). These are the smallest functional units of the breast and over proliferation of epithelial cells covering the breast ducts can result in cancer[5,6]. These cells can attack surrounding healthy tissues through the blood and lymphatics, leading to secondary metastasis[7]. Since breast cancer clinical trends differ for each patient, determining the patient's final perspective is impossible. Thus identification of factors that can predict patient prognosis would be beneficial for clinical and treatment strategy decisions[8]. According to recent statistics published by the Iranian Ministry of Health and Medical Education, the rate of breast cancer is 27 per 100,000 women[9]. It has also been shown that most Iranian women with breast cancer are younger compared to patients in western countries[4].

To determine the stage of breast cancer, the TNM Classification of Malignant Tumors (TNM) system was utilized. In this system, tumor size (T), tumor spread to lymph nodes and lymph node involvement (N) and existence or lack of metastasis (M) are assessed. For instance, it has been revealed that increased tumor size and lymph node involvement are associated with poor prognosis[10]. Based on this system, breast cancer is classified into four stages: stages 1 to 3 are non-metastatic, and stage 4 is usually accompanied by metastasis. This information is beneficial when choosing the appropriate treatment strategy[11].

Motility and invasion of cancer cells are essential for tumor cell metastasis, which requires cytoskeleton actin regulation[12,13]. Cellular actin assembly is reported to be effective on cell migration, motility, attachment, tumor invasion and metastasis[14]. Gelsolin, which plays a significant role in actin assembly, is observed in almost all mammalian tissues. Actin assembly is mainly due to the growth and reforming of apoptotic cells[15]. This protein is coded by the GSN gene located on 9q33 and alterations in gene expression affect general cytoskeleton changes during differentiation and oncogenesis[16]. Gelsolin can act upstream of variable signaling cascades and affect the coordinated regulation of some signaling pathways. Moreover, studies have identified new areas in cancer signal transduction mediated by Gelsolin. Research has indicated the role of erbB-2/EGFR in cancer prognosis among positive tumor patients. Interactions between Gelsolin activation and erbB-2/EGFR) Erythroblastic Leukemia Viral Oncogene Homolog 2/ Epidermal Growth Factor Receptor) have been confirmed via clinical studies and multiple cell culture systems. Furthermore, activation of GTPase, Ras and Rac are crucial in order to mediate motility in downstream processes, and it should be mentioned that Gelsolin quantity regulates Rac expression[17]. Various studies have revealed that Gelsolin is not only a tumor suppressor but also a regulating cytoskeleton factor which controls cell motility and inhibits tumor growth. It has been indicated that the overexpression of Gelsolin can restrict metastasis via attachment to actin molecules. Variation in Gelsolin expression during differentiation and carcinogenesis also affects cytoskeleton changes[12,18]. In other studies, Gelsolin has been introduced as a tumor activator; therefore, it is assumed that recognizing Gel-

solin as a tumor suppressor or activator depends significantly on the type of cancer[19]. This study focused on evaluation of Gelsolin gene expression and its correlation with clinical and demographic findings in Iranian breast cancer patients.

METHODS:

Population and tissue specimen:

In this study, 70 breast cancer patients who visited Imam Khomeini hospital between 2013 and 2014 were evaluated. Samples were obtained from Tumor Bank of Iran, Cancer Institute, Imam Khomeini Hospital Complex. Each sample was then stored at -80 °C for further procedures, which are outlined as follows.

RNA isolation and cDNA synthesis:

RNA extraction from each sample was carried out using a TriPure Isolation Reagent Kit according to the manufacturer's instructions (Roche, Germany). Analysis of RNA quantity was done spectrophotometrically and cDNA was synthesized via oligo dT and random hexamer primers using a RevertAid Kit (Thermo Fisher Scientific). RNA and primers were briefly incubated for 5 minutes at 65°C. After the mixture had cooled, the reverse transcription step was carried out using RevertAid enzyme, dNTP, buffers and RNAase inhibitor for 60 minutes at 42 °C, finishing with incubation at 65°C for 5 min. The Glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) was used as the housekeeping gene. Sequences of Gelsolin and GAPDH mRNAs were obtained from the NCBI database (their Gene ID in NCBI are 2934 and 2597, containing 42 and 10 exons respectively). Afterwards, desired primers were designed using Primer 3 software (**Table 1**). Quantitative Real Time-PCR was performed using RealQ Plus 2x Master Mix Green – (Ampliqon, Denmark). Quantitative Real-Time PCR reactions were performed in Rotogene Q (Qiagene, Hilden, Germany) in 20 µL of PCR master mix containing 10 µL of SYBR-Green QPCR Master Mix, 1 µL of primer, 1 µL of RT products and 8 µL of RNase free water. All tests were run in triplicate to minimize experimental error. Samples with Ct>37 were excluded from the analysis.

Statistical analysis:

Statistical analyses were carried out using SPSS 22.0. (SPSS, Chicago, IL, USA) and Prism 5.0 software. Data was expressed as the mean±SD of at least three independent experiments, and values of $P<0.05$ were considered statistically significant. After obtaining Ct from real-time PCR gene relations, the data was calculated via the $2^{-\Delta Ct}$ formula and assessed using clinical parameters among breast cancer patients. The normality of the data was evaluated using the Kolmogorov–Smirnov distribution and comparison between groups was performed using unpaired student's t test or ANO-

Table 1. Primer sequences for GSN and GAPDH target genes.

Primer Name	Sequence (5´→ 3´)	Size (bp)	Tm°C
GSN Forward	CCTGGGCTTGTCCTACCTTT	155	54.5
GSN Reverse	TGGAACCTTCGATTCTCCAG		53.8
GAPDH Forward	GAAGGTGAAGGTCGGAGTCA	109	53.6
GAPDH Reverse	AATGAAGGGGTCATTGATGG		53.3

VA ($P \leq 0.05$).

RESULTS:

Clinical and pathologic characteristic of patients:

The Imam Khomeini hospital tumor bank provided 70 tumor samples, as well as adjacent tissues as normal tissue samples. Patients' clinical and pathologic characteristics are presented in **Table 2**. The results for TNM stage I, II and III are 3, 38 and 29 respectively. 47% (n=33) of patients were less than 50 years old while 53% (n=37) of them were more than 50 years old. Based on histological grading, the number of patients with grade 2 (n=37) were more than grade 1 (n=9) or 3 (n=24).

Levels of Gelsolin gene expression among normal and tumor tissues:

It was shown that Gelsolin gene expression increased 68.57% among tumor tissues in comparison with normal tissues. (**Fig.1**). Additionally, there was no significant relation between tumor size (P = 0.8957) and patient age (P = 0.3934).

The relation between levels of Gelsolin gene expression and lymph node involvement:

In this study, breast cancer patients were separated based on lymph node involvement. Results indicated that levels of Gelsolin expression were higher among subjects with no lymph node involvement. There was a significant relation between Gelsolin gene expression and lymph node involvement (P= 0.0167) (**Fig.2**).

The relation between levels of Gelsolin gene expression and disease stage:

Cancer patients were classified into four groups based on their disease stage. In this study, group IV was not included. Due to the small studied sample size, groups I and II were merged as one group and patients with stage III were regarded as an independent group. Based on the results, a significant relation between Gelsolin

Table 2. Clinical characteristics of 70 patients with breast cancer. (N= Regional lymph nodes)

Variable	Number	
Lymph node status	N0	32
	N	38
TNM staging	I	3
	II	38
	III	29
Histological grading	1	9
	2	37
	3	24
Tumor size	T1	5
	T2	47
	T3	18
Age	<50 years	37
	≥ 50 years	33

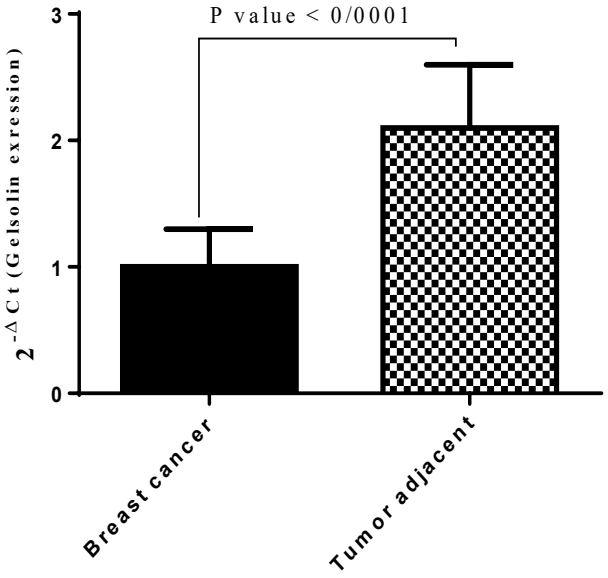

Figure 1. **Relative gene expression of GSN between control and patients group**. mRNA levels GSN were measured using real time PCR. GSN gene expression among tumor tissues is increased 68.57% in comparison with normal tissues. Data are represented as mean ±SEM of at least three separate experiments.

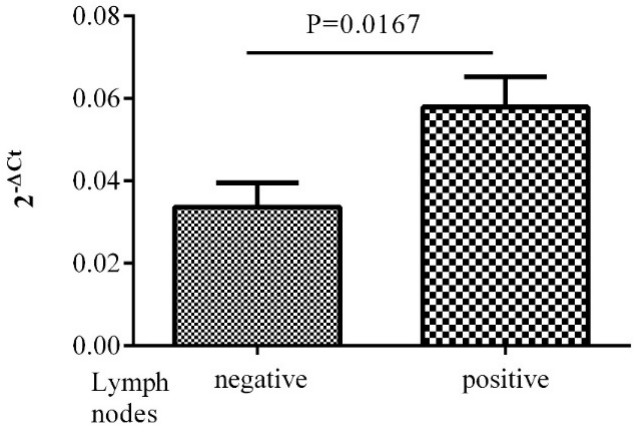

Figure 2. **GSN gene expression and lymph nodes involvement.** mRNA levels of GSN were measured using real time PCR between lymph nodes negative and lymph nodes positive. GSN gene expression among lymph nodes positive was 1.5 fold than lymph nodes negative. Data are represented as mean ±SEM of at least three separate experiments

gene expression and disease stage was observed. (P= 0.0086). (**Fig.3**)

The relation between Gelsolin expression levels and tumor grade:

According to pathological characteristics, tumors were classified into three grades in order to better determine clinical approaches. The results showed no significant relation between levels of Gelsolin expression and tumor grade (P = 0.7848) **(Fig.4)**.

The relation between Gelsolin levels and tumor size:

Tumor size as an indicator of tumor growth, is utilized

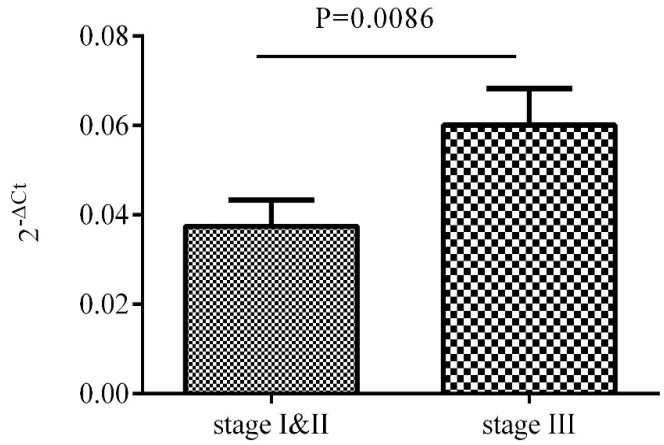

Figure 3. **GSN gene expression and disease stage.** mRNA levels of GSN were measured using real time PCR between stage I, II and III. Patients in stage III had higher expression GSN in comparison to stage I, II group. Data are represented as mean ±SEM of at least three separate experiments

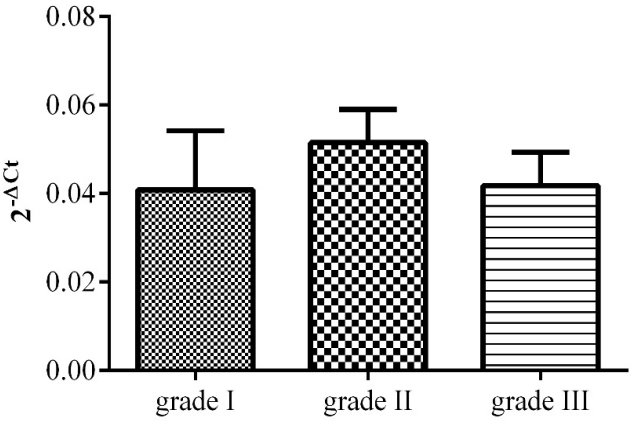

Figure 4. **GSN gene expression and tumor grade.** mRNA levels GSN were measured using real time PCR between three different grads. Data are represented as mean ±SEM of at least three separate experiments

by pathologists for classification. For this study, tumors were classified into three sizes as follows: tumors smaller than 2 cm, tumors between 2 and 5 cm, and tumors larger than 5 cm. The ANOVA results demonstrated that there was no significant relation between Gelsolin expression level and tumor size **(Fig.5)**.

The relation between Gelsolin levels and patient age:

The effect of age on breast cancer patients depends on the family history of the disease and the individual's

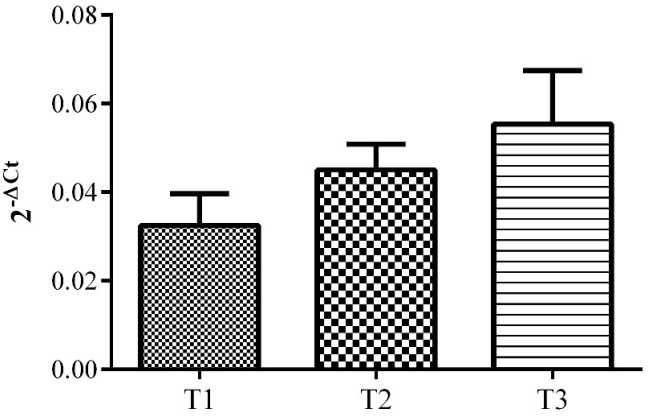

Figure 5. **GSN gene expression and tumor size.** mRNA levels GSN were measured using real time PCR between three different tumor size. Data are represented as mean ±SEM of at least three separate experiments

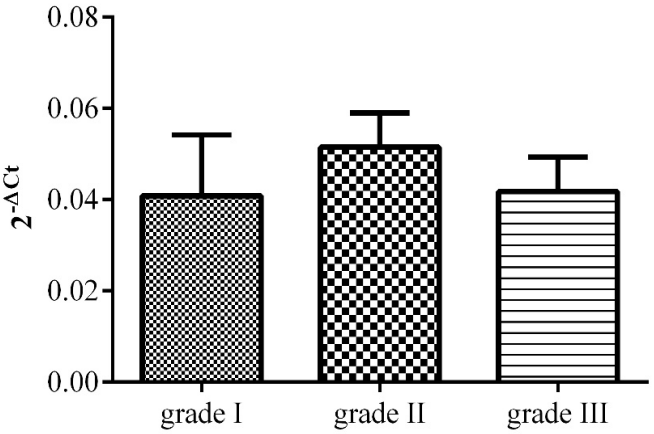

Figure 6. **GSN gene expression and patient's age.** mRNA levels GSN were measured using real time PCR between two different patient's age. Data are represented as mean ±SEM of at least three separate experiments

genetic arrangement. The subjects' age ranged from 31 to 79 years in this study. The results revealed that there was no significant relation between Gelsolin expression levels and tumor size **(Fig.6)**.

DISCUSSION:

Gelsolin is capable of disassembling, capping and assembling actin fibers and subsequently regulating the cytoskeleton dynamically. In addition to playing a role in certain important signaling pathways, it was also revealed that Gelsolin takes part in motility, apoptosis, proliferation, differentiation, epithelial-mesenchymal transition (EMT) and cancer phenotype indication. However, its action during malignancy initiation and progression is still unclear. Even though in most cancer types Gelsolin expression is inhibited, increased expression has been reported in gynecologic cancers. Therefore Gelsolin acts as both an activator and an inhibitor of apoptosis. This contradictory phenomenon has been confirmed in a variety of cancer types[19-21]. Gelsolin expression decreases among pre-malignancy tumors, while its expression rises among higher-grade tumors. Additionally, low levels of Gelsolin expression were also reported to be associated with worse prognosis in epidermal growth factor receptor-positive (EGFR+), erb-b2 receptor tyrosine kinase 2-positive (HER2+) breast cancer, which led to hypothesizing a role for Gelsolin in cell motility and invasion[22]. Since modifying Gelsolin's role in tumor progression is feasible; therefore, this protein might cooperate with other oncogenic factors to accelerate tumor progression[23].

The results of our study showed overexpression of Gelsolin in nearly 68% of patients. In 2015, Runzhi Deng et al. investigated the effect of Gelsolin on the proliferation and motility of Tca 8113 oral carcinoma cells. Their results demonstrated that Gelsolin overexpression considerably enhanced the proliferation and apoptosis of Tca8113 cells. They also indicated that the migration and invasion capability of Tca8113 cells was enhanced via Gelsolin overexpression. As a result, they concluded that higher levels of Gelsolin expression increase cellular growth and motility[20].

In this study, it was revealed that Gelsolin overexpression led to increased lymph node involvement in breast cancer. The expression of this gene was also greater during more progressed stages of breast cancer. Gelsolin is a key regulator of actin disassembly, so its overexpression may lead to cancer cells' motility, affecting the cancer progression trend. Subsequently, it is expected that Gelsolin expression increases along with disease stage. Therefore, an increase in gene expression may be an important factor for breast cancer cell invasion.

In compliance with our results, Wang-Yu Zhu et al. reported in 2012 that an increase in Gelsolin expression was related to lymph node metastasis and progression of disease stage[24]. Also, Abedini et al., showed that in ovarian cancer samples high Gelsolin expression was associated with tumor progression, but there was no correlation between Gelsolin expression, age and tumor grade[21].

Based on our results, there was no significant difference between Gelsolin expression, tumor grade or tumor size. Although Gelsolin expression was higher in younger patients, the difference was not significant, and could be attributed to small sample size. On the contrary, Dar-Bin Shieh et al. examined Gelsolin expression in different stages of oral carcinoma. They found that Gelsolin expression correlated with tumor size, and that expression increased considerably among younger patients[25].

CONCLUSION:

Based on our results, it is assumed that the Gelsolin gene is probably an important factor in breast cancer

as well as being a suitable biomarker for breast cancer clinical anticipation. Thus the pharmaceutical inhibitors of Gelsolin protein can hypothetically restrict the growth and progression of breast tumors. It should be noted that, even though the role of this gene in the invasive behavior of breast cancer cells has been clarified, more information can be obtained about the mechanism of metastasis and its control.

REFERENCES:

1. Banin Hirata BK, Oda JMM, Losi Guembarovski R, Ariza CB, Oliveira CECd, Watanabe MAE. Molecular markers for breast cancer: prediction on tumor behavior. Disease markers. 2014;2014.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. cell. 2011;144(5):646-74.
3. Bray F, Ren JS, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. International Journal of Cancer. 2013;132(5):1133-45.
4. Ghodsi Z, Salehi A, Hojjatoleslami S. Knowledge of Iranian Women about Warning Signs and Risk Factors for Breast Cancer. Procedia-Social and Behavioral Sciences. 2013;93:343-8.
5. Sun X, Sandhu R, Figueroa JD, Gierach GL, Sherman ME, Troester MA. Benign breast tissue composition in breast cancer patients: association with risk factors, clinical variables, and gene expression. Cancer Epidemiology Biomarkers & Prevention. 2014;23(12):2810-8.
6. Sgroi DC. Preinvasive Breast Cancer. Annual review of pathology. 2010;5:193-221.
7. Saleh MS. Reproductive factors and common genetic mutations associated with breast cancer risk in Gaza Strip: Islamic University–Gaza; 2011.
8. Herbein G, Kumar A. The oncogenic potential of human cytomegalovirus and breast cancer. Frontiers in oncology. 2014;4.
9. Jazayeri SB, Saadat S, Ramezani R, Kaviani A. Incidence of primary breast cancer in Iran: Ten-year national cancer registry data report. Cancer Epidemiol. 2015;39(4):519-27.
10. Tan G, Bhoo-Pathy N, Taib N, See M, Jamaris S, Yip C. The Will Rogers phenomenon in the staging of breast cancer–Does it matter? Cancer epidemiology. 2015;39(1):115-7.
11. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. Nature Reviews Cancer. 2005;5(11):845-56.
12. Dos Remedios C, Chhabra D, Kekic M, Dedova I, Tsubakihara M, Berry D, et al. Actin binding proteins: regulation of cytoskeletal microfilaments. Physiological reviews. 2003;83(2):433-73.
13. Cross A, Wilson A, Guerrero M, Thomas K, Bachir A, Kubow K, et al. Breast cancer antiestrogen resistance 3–p130Cas interactions promote adhesion disassembly and invasion in breast cancer cells. Oncogene. 2016.
14. Menhofer MH, Kubisch R, Schreiner L, Zorn M, Foerster F, Mueller R, et al. The actin targeting compound Chondramide inhibits breast cancer metastasis via reduction of cellular contractility. PloS one. 2014;9(11):e112542.
15. Shirkoohi R, Fujita H, Darmanin S, Takimoto M. Gelsolin Induces Promonocytic Leukemia Differentiation Accompanied by Upregulation of p21CIP1. Asian Pacific Journal of Cancer Prevention. 2012;13(9):4827-34.
16. Simoneau M, Aboulkassim TO, LaRue H, Rousseau F, Fradet Y. Four tumor suppressor loci on chromosome 9q in bladder cancer: evidence for two novel candidate regions at 9q22. 3 and 9q31. Oncogene. 1999;18(1):157-63.
17. Li GH, Arora PD, Chen Y, McCulloch CA, Liu P. Multifunctional roles of gelsolin in health and diseases. Medicinal research reviews. 2012;32(5):999-1025.
18. Narayan K, Chumnarnsilpa S, Choe H, Irobi E, Urosev D, Lindberg U, et al. Activation in isolation: exposure of the actin-binding site in the C-terminal half of gelsolin does not require actin1. FEBS letters. 2003;552(2-3):82-5.
19. Baig RM, Mahjabeen I, Sabir M, Masood N, Ali K, Malik FA, et al. Mutational spectrum of Gelsolin and its down regulation is associated with breast cancer. Disease markers. 2013;34(2):71-80.
20. Deng R, Hao J, Han W, Ni Y, Huang X, Hu Q. Gelsolin regulates proliferation, apoptosis, migration and invasion in human oral carcinoma cells. Oncology letters. 2015;9(5):2129-34.
21. Abedini MR, Wang P-W, Huang Y-F, Cao M, Chou C-Y, Shieh D-B, et al. Cell fate regulation by gelsolin in human gynecologic cancers. Proceedings of the National Academy of Sciences. 2014;111(40):14442-7.
22. Stock A-M, Klee F, Edlund K, Grinberg M, Hammad S, Marchan R, et al. Gelsolin is associated with longer metastasis-free survival and reduced cell migration in estrogen receptor-positive breast cancer. Anticancer research. 2015;35(10):5277-85.
23. Zhuo J, Tan EH, Yan B, Tochhawng L, Jayapal M, Koh S, et al. Gelsolin induces colorectal tumor cell invasion via modulation of the urokinase-type plasminogen activator cascade. PloS one. 2012;7(8):e43594.
24. Zhu WY, Hunag YY, Liu XG, He JY, Chen DD, Zeng F, et al. Prognostic Evaluation of CapG, Gelsolin, P-gp, GSTP1, and Topo-II Proteins in Non-Small Cell Lung Cancer. The

Anatomical Record. 2012;295(2):208-14.

25. Shieh D-B, Chen I-W, Wei T-Y, Shao C-Y, Chang H-J, Chung C-H, et al. Tissue expression of gelsolin in oral carcinogenesis progression and its clinicopathological implications. Oral oncology. 2006;42(6):599-606.