Journal of Cell and Molecular Research (2025) 17 (1), 60-70 DOI: 10.22067/jcmr.2025.89162.1096

Research Article

Investigating the Effect of Nano-Lactoferrin on Cytotoxicity and Expression of Autophagy Genes *Beclin 1*, *p53* and *LC3I* in Breast Cancer Cell Line MCF7 and Normal Cell Line Vero

Najibeh Akbari Nowzari [1], Fatemeh Moradian[1*], Ayoub Farhadi[2]

[1] *Department of Basic Sciences, Sari Agricultural Sciences and Natural Resources University, Mazandaran, Iran*
[2] *Department of Animal Sciences, Faculty of Animal and Fishery Sciences, Sari Agricultural Sciences and Natural Resources University, Mazandaran, Iran*

Received 9 September 2024 Accepted 3 September 2025

Abstract

Autophagy can play an inhibitory role in the early stages of cancer, so studying autophagy genes can aid in developing therapeutic strategies. This study investigated the effect of lactoferrin (Lf) and nano-encapsulated Lf (NLf) on the expression levels of autophagy-inducing genes Beclin 1, p53 and LC3I. We also examined the effect of Lf and NLf on the growth rate of breast cancer cell and normal cell lines (MCF7 and Vero). The results showed that the survival percentage of MCF7 cells in NLf treatment was lower than Lf with the same concentrations. The survival of Vero cells treated with NLf and Lf was higher than that of MCF-7 cancer cells, and cell death in this normal cell line was minimal.The level of the Beclin 1gene expression in concentrations of 200 and 300 µg of NLf and Lf increased significantly compared to the control in cancer cells. The expression levels of p53 and LC3I genes were significantly increased in all concentrations of NLf and Lf compared to the control in cancer cells. The expression levels of all three genes in different concentrations of NLf in normal cells showed a significant increase. The level of gene expression and cell death by NLf was slightly higher than that of Lf in cancer cells, which suggests that the intracellular penetration of NLf was greater than Lf. Lf causes cell survival and protects cells during stress by inducing autophagy in normal cells and removing damaged organelles, and causes cell death in cancer cells in the early stages.

***Keywords*:** Autophagy, Biological drug, Breast cancer, Gene expression, Nano-lactoferrin

Introduction

Estimates from the International Agency for Research on Cancer (IARC) reported nearly 20 million new cancer cases and 9.7 million cancer-related deaths in 2022. Female breast cancer accounted for 11.6% of cancers in the year 2022 (Bray et al., 2024). Breast cancer is the second most common cancer in the world and is the most common cancer in women in Iran (Khodavirdi et al., 2019; Roshandel et al., 2021). According to the International Agency for Research on Cancer in Iran, 12.9% of cancers were related to breast cancer in 2020 (Zendehdel, 2020). Despite the various treatment methods, due to side effects and the resistance of cancer cells to the treatment, there is a need for targeted treatments. Autophagy is known as a vital process in cells that plays a role in biological evolution, the immune system and cell death (Hosseini et al., 2013). Autophagy is activated during unfavorable conditions in the cell, such as starvation, hypoxia, DNA damage, infection with microorganisms and pathogens, and oxidative stress in the cell (Chmurska et al., 2021). Autophagy, which destroys redundant or damaged cellular components, has been implicated in a variety of human diseases, including cancer. Autophagy affects cancer initiation and progression differently due to the inherent overlap of autophagy and cancer signaling pathways (Shi et al., 2013). Autophagy is a tumor suppressor in the early stages of tumorigenesis, as decreased autophagy was found in tumor cells and may be associated with malignancy. In this case, the induction of autophagy seems to be useful for cancer prevention. However, in advanced tumors, autophagy can be tumor-promoting, and cancer cells can use autophagy to survive under metabolic stress (Choi., 2012; Karabi et al., 2022). p53 as a tumor suppressor gene prevents the development of cancer. p53 can induce autophagy. P53 protein can direct transcription of autophagy-related proteins and indirectly activate autophagy using inhibition of the mTOR signaling pathway (Taji et al., 2017). The P53 protein exists in two

* Corresponding author's e-mail address: f.moradian@sanru.ac.ir

forms: cytoplasmic and nuclear. Within the nucleus, p53 can function as a transcription factor regulating autophagy-inducing genes (Maiuri et al.,2009).

Beclin 1 protein is a protein that interacts with Beclin 2 protein and PI3K class 3 and can have an important role in regulating autophagy and cell death. Inactivation of Beclin 1 caused tumor expansion in mice, and Beclin 1 gene expression led to the inhibition of breast tumor formation in mouse models (Taji et al., 2017). Beclin 1 is an important protein related to autophagy in human cells, which is involved in cell death and survival and is widely expressed in normal mammary epithelial cells (Li et al., 2010). LC3 protein is one of the important and necessary components for the autophagosome membrane, and after activation, it causes autophagy induction (Davoodi.,2019; Dikic et al., 2018). Decreased LC3I expression increases the risk of distant metastasis in triple-negative breast cancer (TNBC) (He et al., 2013). Lactoferrin (Lf) is a glycoprotein that has antibacterial, antiviral, anticancer, and immune system-regulating properties. Lf has several beneficial effects on human health (karabi et al., 2022). It has been proven that this natural protein is a promising biological drug in anticancer research, and Lf can regulate multiple signalling pathways to inhibit cancer cells (Kanwar et al., 2015). Since Lf and its derivatives are also found in mammalian milk, they are non-toxic and can be suitable alternatives and complements to current anticancer drugs due to their anticancer activity (Zhang et al., 2016). Lf has anticancer activity with different mechanisms of action, which, depending on the type of cancer, can induce apoptosis, cell cycle arrest, have antiangiogenic effects, and inhibit metastasis (Moreno-Expósito et al., 2018). Other mechanisms for the antitumor role of lactoferrin have also been identified, such as regulation of natural killer cell activity and regulation of the expression of G1 phase proteins (Grosso et al., 2019). Lf stopped the growth of human mammary gland carcinoma cells between G1 and S phases (Adlerova et al., 2008). Lf has shown positive effects in increasing autophagy activity through AMPK signaling via the LRP1 receptor (Grosso et al., 2019). Natural products and bioactive compounds that can modulate autophagy have attracted the attention of researchers for the treatment of diseases, including cancer, of which lactoferrin is an example due to its multiple biological functions (Karabi et al., 2022).

The biological properties of Lf are mediated by specific receptors on the surface of target cells. These receptors are found on mucosal epithelial cells, liver cells, monocytes, macrophages, polymorphonuclear leukocytes, lymphocytes, thrombocytes, and fibroblasts (Adlerova et al., 2008). A wide range of cell receptors recognizes Lf. These receptors play a role in many cells signaling processes and may play a role in cancer and other diseases (Watanabe et al., 2017). Most protein drugs in the body have limitations, such as high molecular weight that prevents them from passing through the tissues, and also have a short half-life and enzyme digestion, which makes their transfer difficult. Nanocarriers for drug delivery have unique features like greater drug efficacy, less toxicity, and encapsulation ability and carry poorly soluble substances (Moradian et al., 2023). For the improvement of the therapeutic effect of Lf protein for cancer treatment, a nano-formulation with a higher absorption capacity in the live system is used. Different types of nanoparticles were prepared as carriers of Lf protein for use in various cancers (Kanwar et al., 2015). Functionally beneficial properties of Lf may be reduced during processing in the living system due to changes in its structure. Therefore, to improve the stability of the active molecule of Lf and its absorption in the small intestine and to control its release, the nano-encapsulation method should be used. Today, in addition to the common treatment methods, the study of autophagy, a type of cell death, has received attention in the specific treatment of cancer. In the present study, the effect of encapsulated NLf and Lf on the survival rate of breast cancer cells and normal cells, as well as the effect on the expression of genes involved in autophagy, including Beclin 1, p53 and LC3I in breast cancer cell line MCF7 and normal cells, was studied. Given the importance of specific therapy by inducing cell death through the autophagy pathway, fundamental studies on these genes and anticancer compounds (Lf) that induce these genes can pave the way for future therapeutic strategies.

Considering the anticancer effects of Lf protein and its mechanism of action by inducing cell death, one of the objectives of this study was to examine the effect of NLf and its comparison with Lf in inducing autophagy-type cell death, with the effect on the expression of genes involved in inducing autophagy.

Materials and Methods

Preparation of nano-chitosan and encapsulation of Lf

2.5 mg/ml of chitosan (the low molecular weight polymer CS, Sigma) was dissolved in 1% acetic acid solution and tripolyphosphate (TPP), with 1 mg/ml dissolved in deionized water. Lf was dissolved with

a 0.2 mg/ml concentration in TPP buffer. TPP buffer containing Lf was added drop by drop to chitosan at a rate of 8 ml/min and continuously stirred at 1000 rpm on a stirrer at room temperature for two hours until a homogeneous solution was obtained (Hedyeloo et al. 2023). It was kept under UV light for 30 minutes to sterilize NLf.

Cell culture and MTT assay

Human breast cancer cell line MCF-7 and normal cell line Vero were purchased from the Pasteur Institute (Iran). The cells were cultured in RPMI-1640 with 20% FBS and incubated at 37 °C with 5% CO_2. After the growth of the cells and reaching 80% confluency, the cells were transferred to a 96-well plate so that there were 1×10^4 cells in each well and incubated for 24 hours. Then, the different concentrations of Lf and NLf 0, 100, 200, and 300 µg/ml were treated with three replicates and incubated for 24 hours. Then 50 microliters of MTT solution were added to each well and placed in an incubator at 37°C for 2-4 hours until purple crystals formed. After the formation of crystals, the supernatant liquid was removed, and 150 microliters of acidic isopropanol was added to the wells, placed in the dark for 30 minutes, and then analyzed using an ELISA reader (Awareness Tchnology Stat Fax 2100, USA) at a wavelength of 490 nm.

Treatment with Lf and NLf

MCF7 and Vero cells were cultured on two 6-well plates with a number of $2x10^4$ cells per well, and after appropriate growth, the cells were treated with four concentrations of 0, 100, 200, and 300 µg/ml of NLf and Lf with three repetitions. Then they were incubated for 24 hours.

RNA extraction and cDNA synthesis

Total RNA was extracted using a total RNA extraction kit (Parstous, Iran) according to the kit's method. To ensure that the RNA was not degraded, some of the RNA was electrophoresed on a 1% agarose gel to confirm its purity. cDNA was synthesized using the Easy cDNA synthesis kit of Parstos company (Iran). To measure the concentration of synthesized cDNA, its absorbance was read by a spectroscopic device (Bio WAVE-Pharmacia, USA) at OD260 and 280 nm.

Design of dedicated primers

The nucleotide sequences for Beclin 1, p53 and LC3I genes were extracted from the NCBI database (https://www.ncbi.nlm.nih.gov/nucleotide/). The specific primers were designed according to the conserved regions of the genes and without reporting SNPs (https://www.ncbi.nlm.nih.gov/snp) using Gene Runner software (version 6.5.52). The β-actin gene was considered as a reference (Khalafi et al., 2020). The sequences of primers were; Beclin 1 (forward): 5'-GCACAGTGGACAGTTTGGCACAATC-3', reverse: 5'-CTCCAGGAAAGCCACCATTGCATG-3'; p53 (forward): 5'-CCTGTGCAGCTGTGGGTTGATTC-3', reverse: 5'-GGCACCACCACACTATGTCGAAAAG-3'; *MAP1LC3I*(forward): 5'-GGTACAGCAGATCCGCGACCA-3', reverse: 5'-CGTCCTCGTCTTTCTCCTGCTCGT-3'.

Gene expression analysis

The relative expression level of Beclin 1, p53 and LC3I genes after treatment with Lf and NLf was determined using real-time PCR. The real-time reactions were prepared using a YTA SYBR Green qPCR Master Mix 2X kit (Yekta Tajhiz Azma Company, Iran), and it was done in a real-time PCR device (Corbett Rotor Gene 3000, Australia). The real-time program included initial denaturation at 95 °C for 5 min, followed by a second denaturation at 95 °C for 1 min, annealing at 60 °C for 30 sec, and extension at 72 °C for 45 sec (by repeating 35 cycles), and the final extension at 72 °C for 5 min.

Data analysis

The relative expression level of Beclin 1, p53 and LC3I genes was measured by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Statistical analysis was performed in SAS statistical software, version 9.1, with Duncan's multiple-range test, and the significance of the data was considered with $P<0.05$.

Study of biochemical pathways of p53 and LC3I genes in the KEGG database

Biochemical pathways of the p53 gene in breast cancer and the LC3I gene in autophagy were extracted from the KEGG database (www.genome.jp/pathway).

Results

MTT assay and determining the survival rate of cancer and normal cells

The survival percentage of the MCF7 cells treated with NLf at concentrations of 100, 200, and 300 µg/ml was 61.3, 45.5, and 30.68%, respectively, and for Lf was 90, 86, and 80%, respectively. The percentage of survival in the Vero cells treated with concentrations of 100, 200, and 300 µg/ml of NLf was 100, 96.1, and 93%, respectively, and for Lf was

100, 99, and 96%, respectively. The percentage of survival in the treatment with concentrations of 100, 200, and 300 µg/ml of chitosan in both cancer cells and normal cells was equal to 100% and showed that it does not have a lethal effect on the cells (Figures 1 and 2).

Gene expression level

The expression level of the p53 gene in the MCF7 cells in the treatment with concentrations of 100, 200, and 300 µg/ml of NLf was 1.81, 1.84, and 2.07 times, respectively, and in the treatment with Lf, it was 1.62, 1.94 and 2 times, respectively which showed a significant increase in both treatments compared to the control ($P<0.05$) (Figure 3 and 4). The amount of Beclin 1 gene expression in the treatment with concentrations of 100, 200, and 300 µg/ml of NLf in MCF7 cancer cells was 0.80, 1.22, and 1.28 times, respectively, and in the treatment with Lf, it was 0.77, 1.18, and 1.14 times, which showed a decrease in the concentration of 100 µg/ml and a significant increase in the other two concentrations ($P<0.05$) (Figure 3 and 4).

The amount of the LC3I gene expression in the treatment with concentrations of 100, 200, and 300 µg/ml of NLf in MCF7 cancer cells was 1.27, 2.54, and 2.34 times, respectively, and in the treatment with Lf, it was 1.09, 1.75, and 1.74 times, respectively, which showed a significant increase compared to the control in all concentrations ($P<0.05$) (Figure 3 and 4).

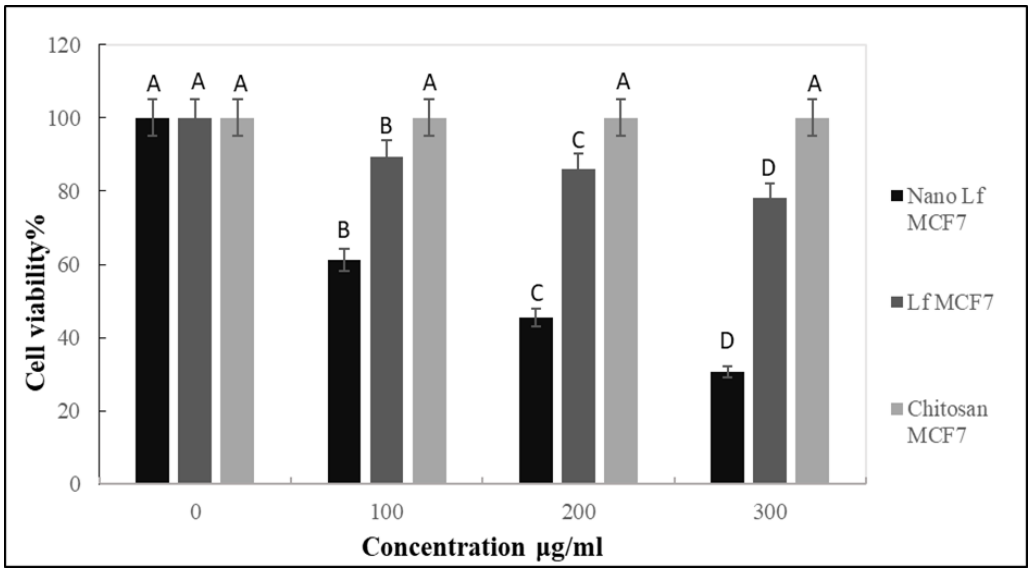

Figure 1. Graph of the survival percentage of the MCF7 cell line in treatment with concentrations of 100, 200, and 300 µg/ml of NLf, Lf, and chitosan.

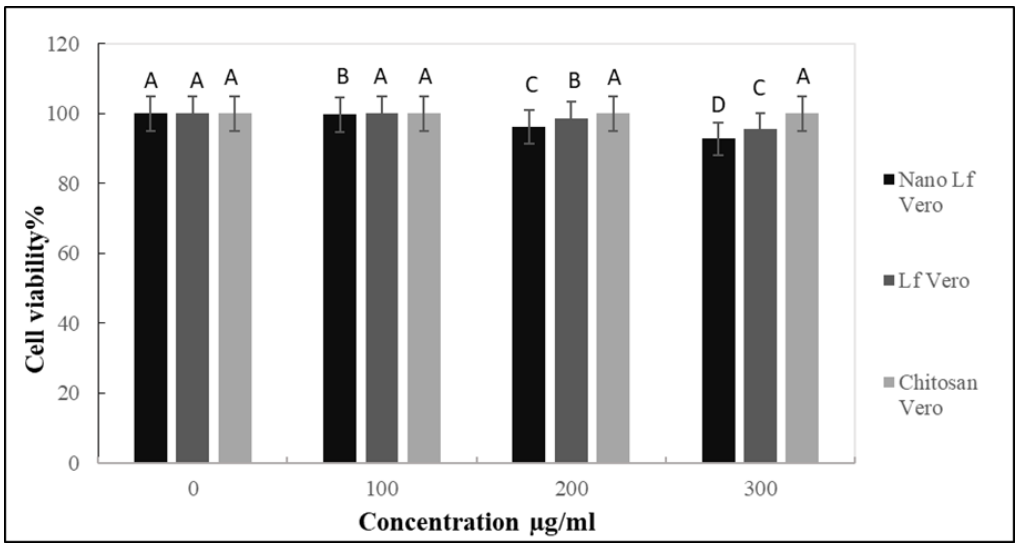

Figure 2. Graph of the survival percentage of the Vero cell line in treatment with concentrations of 100, 200, and 300 µg/ml of NLf, Lf, and chitosan.

In the Vero cell line, the amount of the p53 gene expression in the treatment with concentrations of 100, 200, and 300 µg/ml of NLf was 1.05, 1.44, and 1.55 times, respectively, which was significantly increased in two concentrations of 200 and 300 µg/ml compared to control (P<0.05) (Figure 5). The LC3I gene expression level in the treatment with concentrations of 100, 200, and 300 µg/ml of NLf was 1.28, 1.67, and 1.76 times, respectively, in normal cells, which showed a significant increase compared to the control (P<0.05) (Figure 5). The expression level of the Beclin 1 gene in the treatment with concentrations of 100, 200, and 300 µg/ml of NLf was 1.22, 2.77, and 2.80 times, respectively in normal cells, which showed a significant increase compared to the control (P< 0.05) (Figure 5).

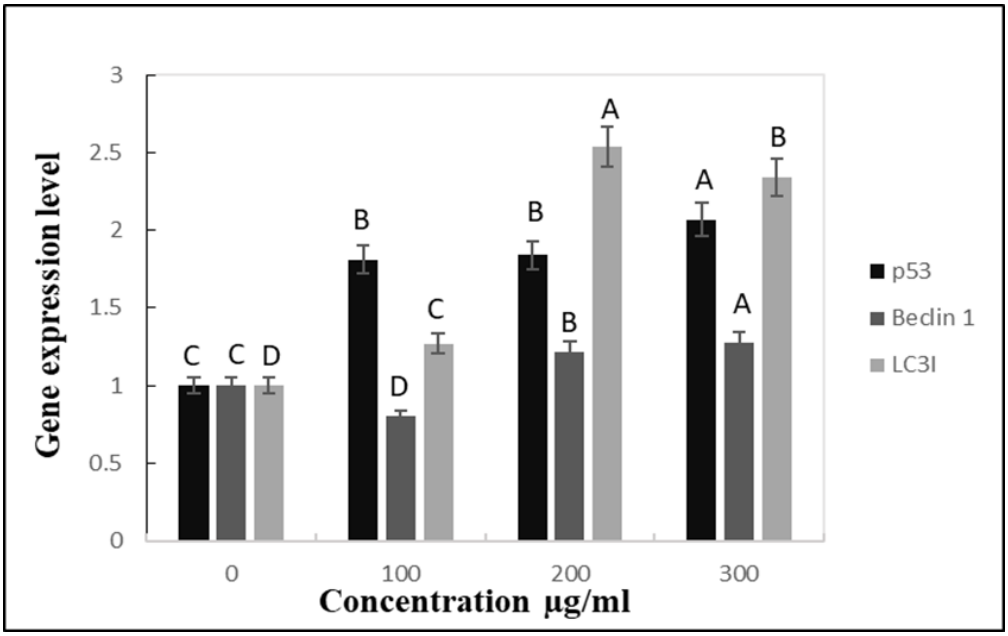

Figure 3. The graph related to the relative expression level of p53, LC3I and Becline 1 gene in the MCF7 cell line in treatment with concentrations of 100, 200, and 300 µg/ml of NLf.

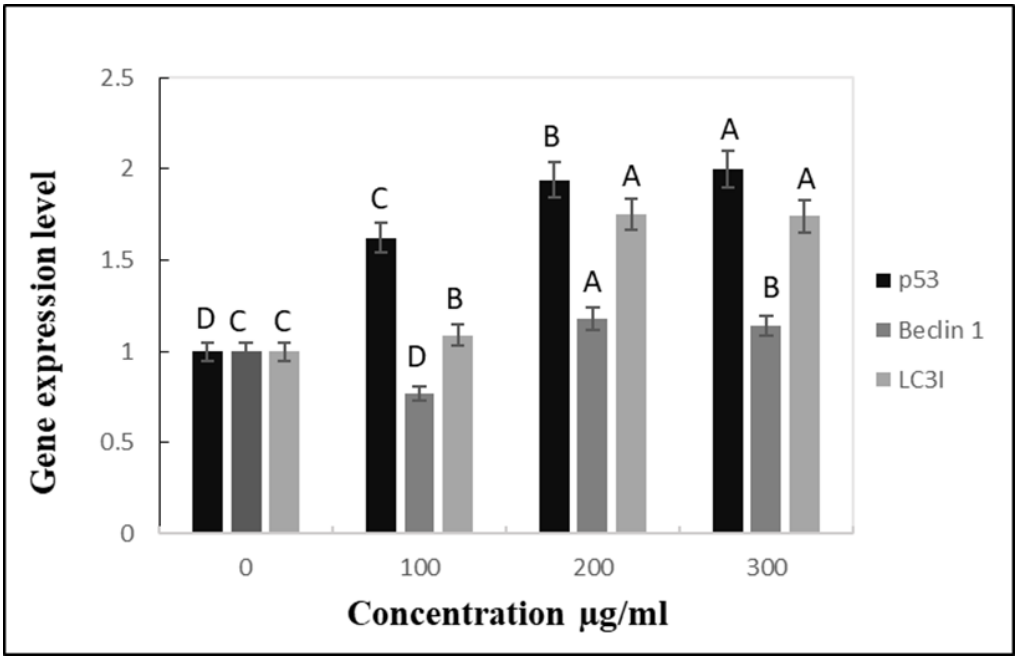

Figure 4. The graph related to the relative expression level of p53, LC3I and Becline 1 gene in the MCF7 cell line in treatment with concentrations of 100, 200, and 300 µg/ml of Lf.

Study of biochemical pathways of p53 and LC3I genes

When the cell DNA is damaged, a mutation in the p53 gene and its normal inactivity occurs, the expression of the inhibitor of cyclin kinase p21 is reduced and uncontrollable proliferation occurs in the cell. On the other hand, the decrease in the expression or non-expression of the p53 gene causes a decrease in the amount of expression of the genes involved in apoptosis, Bax and Bak, as a result of stopping apoptosis and increasing the survival of cancer cells. In addition, it deactivates p48 and POL4 and creates genomic instability, which plays a role in causing cancer (Figure 6).

When oxidative stress, such as reactive oxygen species (ROS), occurs in the cell, or when cellular energy levels are low and ATP decreases, ATG12 is activated by ATG7 and subsequently binds to ATG5. Then the conjugation of ATG12-ATG5 complex with ATG16L1 occurs, and the ternary protein complex ATG12-ATG5-ATG16L is formed. Finally, this complex, together with ATG3, conjugates LC3-I with phosphatidyl-ethanolamine and LC3-II is produced. LC3II remains on the autophagosome membrane, which completes the autophagy process and merges the autophagosome with the lysosome membrane. Then, this complex with ATG3 causes conjugates LC3-I with phosphatidyl-ethanolamine, and LC3-II is produced.

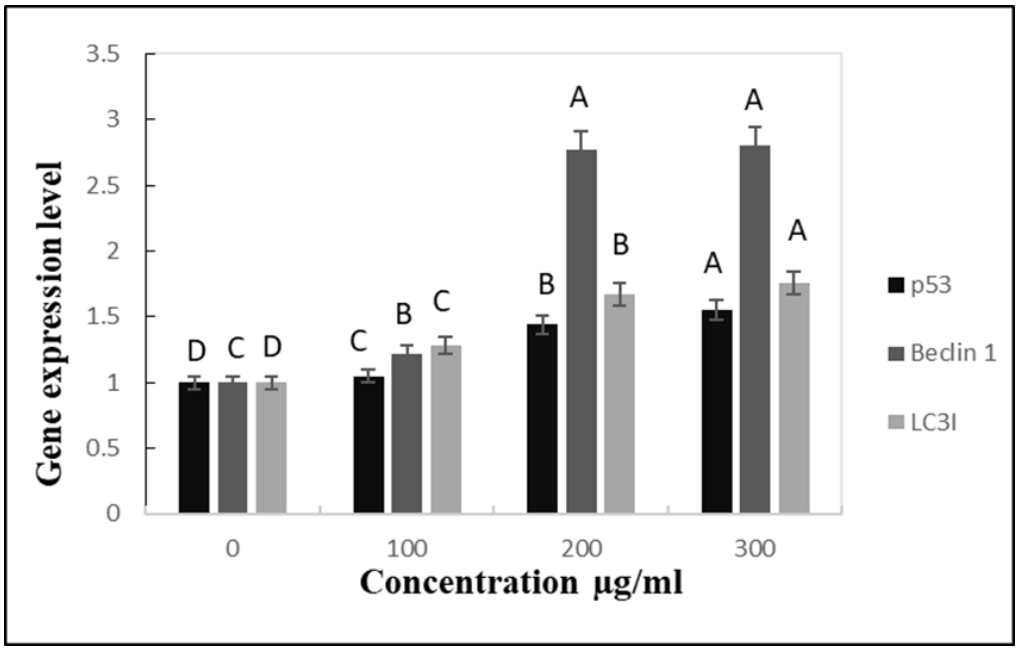

Figure 5. The graph related to the relative expression level of p53, LC3I and Becline 1 gene in the Vero cell line in treatment with concentrations of 100, 200, and 300 µg/ml of NLf.

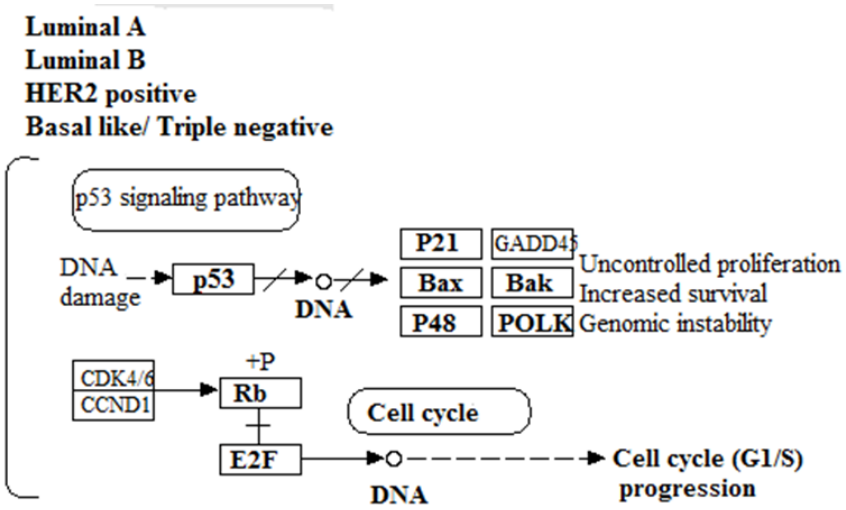

Figure 6. Biochemical pathway of the p53 gene in breast cancer in the KEGG database.

LC3II remains on the autophagosome membrane, which completes the autophagy process and merges the autophagosome with the lysosome membrane (Figure 7).

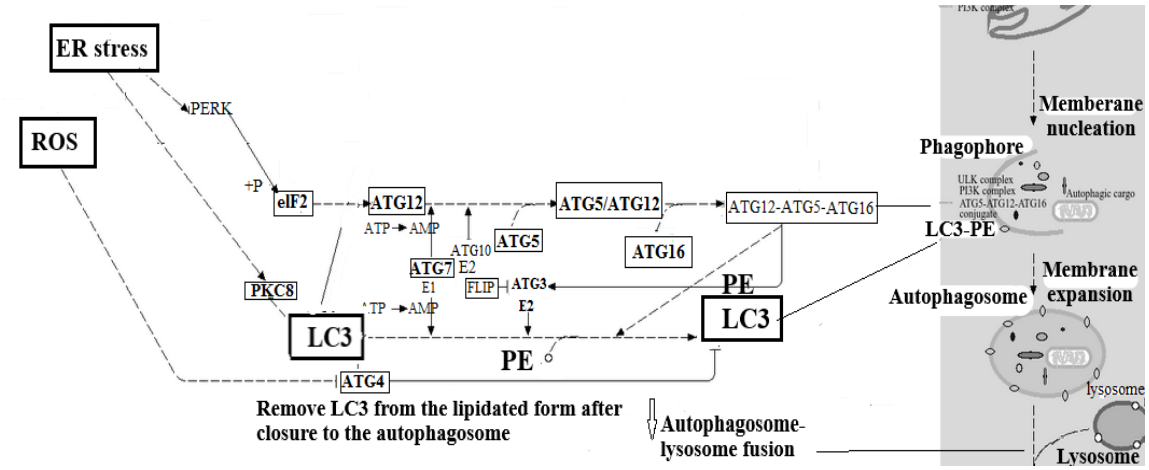

Figure 7. The LC3I gene regulatory pathway in autophagy (https://www.genome.jp/pathway/hsa04140).

Discussion

Breast cancer is one of the most common cancers in women around the world (Jafari et al., 2018). In addition to conventional treatment methods, targeted therapies that induce autophagy-mediated cell death have been explored for the specific treatment of cancer (Mashhadi Kholerdi et al. 2024). Lf, as a protein with food-pharmaceutical properties having various important roles, has attracted the attention of many researchers. The mechanism of autophagy regulation by Lf and its anticancer role can be significant for the complementary treatment of breast cancer (Mashhadi Kholerdi et al. 2024). In the present study, different concentrations of bovine Lf and NLf were used to evaluate their growth inhibition effect in breast cancer cells MCF7 as well as normal Vero cells. The results showed that Lf and NLf reduced breast cancer cell growth in a dose-dependent manner. NLf could reduce cancer cell viability more than Lf. At the same concentrations, they showed a mild lethal effect in normal cells, suggesting that this lethality was due to the induction of the cell's natural programmed cell death mechanism. In a previous study, esophageal cancer cells KYSE-30 and gastric cancer cells AGS were treated with different concentrations of Lf, and the cell survival rate after 20 hours was 83 and 66%, respectively, but it had no effect on the growth of the normal cells HEK and HFF (Farziyan et al., 2015; Amiri et al., 2016). Bovine Lf (bLf) caused growth inhibition in breast cancer cell lines T-47D, MDA-MB-231, Hs578 T and MCF7 in a concentration-dependent manner (Zhang et al., 2014).

Oral treatment of Lf nanoparticles coated with chitosan in mice that developed tumors caused a significant reduction in tumor size after 35 days (Kanwar et al., 2015). In the study of the pharmacokinetics and bioavailability of oral Lf in colon cancer, it was shown that NLf was present in tumor cells after oral feeding of mice with PEG/PLA-encapsulated NLf, which entered the tumor more than pure Lf, indicating an increased capacity to transport Lf when nano-encapsulated (Kanwar et al., 2015). Autophagy is recognized as an important cellular process that can be addressed for targeted cancer therapy by identifying key regulators of autophagy. Autophagy can be important in regulating the growth and progression of cancer and in determining the response of tumor cells to treatment (Hippert et al., 2006). In the present study, the effect of three different concentrations of NLf and Lf on the expression of three important genes involved in the autophagy process was investigated. The results showed that NLf and Lf in concentrations of 200 and 300 µg/ml could increase the expression of all three genes, Beclin 1, p53 and LC3I in breast cancer cells. From this result, it can be speculated that autophagy occurs by increasing the expression of these genes, which reduces the growth and proliferation of breast cancer cells, and the viability test also showed cell death. Liang et al. showed that the inhibition of MCF7 cell proliferation is related to the autophagy-inducing activity of Beclin 1 in this cell. In addition, Beclin 1 protein expression is often low in human breast cancer epithelial cell lines and tissues (Liang et al.,1999). The expression of Beclin 1 is significantly decreased in the majority of OTSCC oral squamous cell

carcinoma tissues and five OTSCC cell lines compared to non-cancerous tissues and normal tongue epithelial cell lines, respectively (Hu et al.,2016). Quercetin was able to increase the expression of *LC3I* and induce autophagy, followed by the death of breast cancer cells (Safarian and Erfani, 2021). Nurdinov et al. reported that the expression level of *LC3I* is decreased in cancer cells, and the decrease in *LC3I* level is associated with poor outcomes in breast cancer cells. In particular, *LC3I* expression is low in triple-negative breast cancer (TNBC) cells and is significantly associated with lymph node invasion, the risk of distant metastasis and high mortality in TNBC patients (Nurdinov et al., 2021). Chu et al. investigated allicin-induced autophagic cell death in human hepatocellular carcinoma Hep G2 cells. The results showed that allicin induces P53-mediated autophagy and inhibits the survival of human hepatocellular carcinoma cell lines. They observed that allicin decreased cytoplasmic p53 level, PI3K/mTOR signaling pathway and Bcl-2 level and increased the expression of AMPK/TSC2 and Beclin *1* signaling pathways in Hep G2 cells (Chu et al., 2012).

According to some data, p53 promotes autophagy through activation of DAPK, DNA damage-regulated autophagy modulator 1, and proapoptotic BCL-2 proteins (e.g., BAD, BAX, BNIP3, and PUMA) (Tasdemir et al., 2008). In response to genotoxic stress, p53 can activate the transcription of ULK1 and ULK2, which in turn leads to increased levels of autophagy and contributes to cell death (Gao et al., 2011).

The protective effect of lactoferrin in human renal tubular epithelial cells was investigated, and the results showed that Lf induces autophagy through activation of AMPK and inhibition of the Akt/mTOR pathway (Hsu et al., 2020). Zhang et al. demonstrated that lactoferrin inhibits mTOR signaling in a dose-dependent manner. They showed that bLf increased the levels of phosphorylated AMPKα in a dose-dependent manner in all breast cancer cell lines. Thus, lactoferrin induces autophagy by increasing AMPKα activity and inhibiting mTOR activity (Zhang et al., 2014). The results of this study and other studies confirmed that Lf protein is capable of inducing autophagy by increasing the expression or activity of genes related to autophagy.

The expression level of three genes, Beclin 1, p53 and LC3I, increased in normal Vero cells under the effect of NLf. It can be said that in a normal cell, inducing autophagy, a type of programmed cell death, ensures the survival of the normal cell, by allowing the death of several damaged cells. Of course, it should be kept in mind that the expression level of each gene can change under the influence of different factors, and in some cases, it is also influenced by the expression of other genes that interact with the target gene. Naturally, autophagy is beneficial to the normal cell as it regulates the recycling of aged proteins, removes damaged organelles, and protects cells during starvation (Masini et al., 2009).

Autophagy plays a dual role in cancer, depending on the stage or genetic background of the cancer. In early tumorigenesis, autophagy helps with normal cell metabolism, removes damaged proteins and organelles, and prevents tumor initiation. When tumors reach advanced stages and are subjected to environmental stresses, autophagy can reduce DNA damage, enhance cancer cell survival and resistance to stress, and ultimately increase tumor survival. In advanced stages of cancer, autophagy can lead to resistance to treatment (Li et al., 2020; Chen et al., 2009; Maycotte and Thorburn, 2014). A better understanding of the dual roles of autophagy in cancer biology may help identify or design new drugs that induce or inhibit autophagy (Russo and Russo 2018). Therefore, basic studies can help to better understand the mechanism of autophagy and the function of genes involved in this process.

The database was used to show the functional pathway of the two genes p53 and LC3I in breast cancer and the autophagy process. In fact, showing the biochemical pathway of genes to complete the experimental part of a study can well demonstrate the mechanism of action of genes and how the activity of a gene in a pathway leading to a process can be sensitive. The p*53* signaling pathway can reveal the mechanism of this gene in breast cancer and clearly explains the importance of this gene's function in cancer prevention. The regulatory pathway of the LC3I gene shows the importance of this gene in inducing autophagy. Therefore, environmental factors such as oxidants are able to inhibit the expression of this gene, and antioxidants or compounds that prevent the destructive effects of stress reactions are able to increase the expression and activity of this gene and help normal cells survive in adverse conditions, and also help advanced cancer cells survive. Therefore, it can be considered a hot spot target for the induction or inhibition of autophagy.

Understanding the functional pathway of genes in the process related to cancer development and the occurrence of autophagy can highlight positive points for other researchers to continue and complete the present study. The regulatory pathways of these genes and their points of influence on other genes

could be useful for targeting other genes for the rational design of drugs or cancer diagnostic markers.

Conclusion

Despite the dual role of autophagy in cancer, which depends on the state of the cells and can stop or progress the tumor, autophagy can play an inhibitory role in the early stages of cancer and help in cancer treatment. Considering the important role of p53 and Becline 1, as well as LC3 I in the autophagy induction pathway, these genes can be used as a marker for cancer. In order to develop successful strategies for cancer treatment, we need to study more about the role of autophagy in terms of tumor stages, cell type, and genetic factors, and we need to learn about the specific pathways of autophagy that are activated or inhibited in various cancer treatments. Therefore, the present study and similar studies can be important to achieve this goal. Targeting autophagy-related pathways is a promising tool for the development of new cancer therapies. The fact that the underlying molecular mechanisms and specific targets of autophagy in cancer need to be identified is a major challenge.

Acknowledgment
The authors are thankful for the facility and assistance offered by the Cell and Molecular Biology Lab at Sari Agricultural Sciences and Natural Resources University. A portion of the funding for this research project was provided by the Fatemeh Moradian Grant from the University of Agricultural Sciences and Natural Resources.

Conflict of Interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Adlerova L., Bartoskova A. and Faldyna, M.J.V.M. (2008) Lactoferrin: a review. Veterinarni Medicina 53(9): 457-468.

Amiri F., Moradian F. and Rafiei A.R. (2015) Anticancer effect of lactoferrin on stomach cancer cell line AGS. Research in Molecular medicine 3(2):11-16.

Bray F., Laversanne M., Sung H., Ferlay J., Siegel R.L., Soerjomataram I.and Jemal A. (2024) Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians 1-35. https://doi.org/10.3322/caac.21834.

Chu Y.L., Ho C.T., Chung J.G., Rajasekaran R. and Sheen L.Y. (2012) Allicin induces p53-mediated autophagy in Hep G2 human liver cancer cells. Journal of Agricultural and Food Chemistry 60(34): 8363-8371.

Chen N. and Karantza-Wadsworth V. (2009) Role and regulation of autophagy in cancer. Biochimica et Biophysica Acta (BBA)-Molecular Cel Research 1793(9): 1516-1523.

Choi K.S. (2012) Autophagy and cancer. Experimental and Molecular Medicine, 44(2): 109-120.

Chmurska A., Matczak K. and Marczak A. (2021) Two Faces of Autophagy in the Struggle against Cancer. International Journal of Molecular Sciences 22(6): 2981.

Davoodi N. (2019) The effect of L-carnitine on authophagy in infarcted heart in wistar male rats. Doctoral dissertation, Tabriz University of Medical Sciences, Faculty of Pharmacy.

Dikic I. and Elazar, Z. (2018) Mechanism and medical implications of mammalian autophagy. Nature Reviews Molecular Cell Biology 19(6): 349-364.

Farziyan M.A., Moradian F. and Rafiei A.R. (2016) The study of anticancer effect of bovine lactoferrin on human esophagus cancer cell line. Research in Molecular Medicine 4(1): 1-6.

Gao W., Shen Z., Shang L. and Wang X. (2011) Upregulation of human autophagy-initiation kinase ULK1 by tumor suppressor p53 contributes to DNA-damage-induced cell death. Cell Death Differ. https://doi.org/10.1038/cdd.2011.33.

Grosso R.A., Caldarone P.V.S., Sánchez M.C., Chiabrando G.A., Colombo M.I. and Fader C.M. (2019) Hemin induces autophagy in a leukemic erythroblast cell line through the LRP1 receptor. Bioscience Reports 39: 1.

He J.H., Luo R.Z., Cai M.Y., Li M., Lu J.B. and Yuan Z.Y. (2013) Decreased expression of light chain 3 (LC3) increased the risk of distant metastasis in triple-negative breast cancer. Medical Oncology 30(1): 1-6.

Hedyeloo F., Moradian F., Rostami M. (2023) Nanoencapsulation of oral- pharmaceutical lactoferrin using chitosan and the evaluation of stability against trypsin and pepsin and its

antibacterial effect. (Micro and Nano Leterrs) Micro Nano Letter 18: e12174.

Hippert M.M., O'Toole P.S. and Thorburn A. (2006) Autophagy in cancer: good, bad, or both? Cancer Research 66(19): 9349-9351.

Hsu Y.H., Chiu I.J., Lin Y.F., Chen Y.J., Lee Y.H., Chiu H.W. (2020) Lactoferrin contributes a renoprotective effect in acute kidney injury and early renal fibrosis. Pharmaceutics 12 (5):434. h

Hu Z., Zhong Z., Huang S., Wen H., Chen X., Chu H. and Sun C. (2016) Decreased expression of Beclin-1 is significantly associated with a poor prognosis in oral tongue squamous cell carcinoma. Molecular Medicine Report 14(2): 1567-1573.

Jafari S.H., Saadatpour Z., Salmaninejad A., Momeni F., Mokhtari M., Nahand J.S. and Kianmehr M. (2018) Breast cancer diagnosis: Imaging techniques and biochemical markers. Journal of Cellular Pysiology 233(7): 5200-5213.

Karabi Z., Moradian F. and Kheirabadi M. (2022) The effect of lactoferrin on ULK1 and ATG13 genes expression in breast cancer cell line MCF7 and bioinformatics studies of protein interaction between lactoferrin and the autophagy initiation complex. Cell Biochemistry and Biophysics 80(4): 795-806.

Kanwar J.R., Roy K., Patel Y., Zhou S.F., Singh M.R., Singh D. and Kanwar R.K. (2015) Multifunctional iron bound lactoferrin and nanomedicinal approaches to enhance its bioactive functions. Molecules 20(6): 9703-9731.

Khalafi A., Moradian F., Rafiei A.R. (2020) Anticancer effect of bovine lactoferrin on breast cancer cell line MCF7 and the evaluation of Bax and Bak genes expression involved in apoptosis. Research In Molecular Medicine. Research in Molecular Medicine 8(3):107-116.

Khodavirdi T., Eskandari H., Borjali A. and Farrokhi N. (2019) Explaining the spiritual needs of breast cancer patients: A qualitative study. Middle Eastern Journal of Disability Studies 9: 125-125.

Li Z., Chen B., Wu Y., Jin F., Xia Y. and Liu X. (2010) Genetic and epigenetic silencing of the beclin 1 gene in sporadic breast tumors. BMC Cancer 10(1): 1-12.

Li X., He S. and Ma B. (2020) Autophagy and autophagy-related proteins in cancer. Molecular Cancer 19(1): 1-16.

Liang X.H., Jackson, S., Seaman M., Brown K., Kempkes B., Hibshoosh H. and Levine B. (1999) Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 402 (6762): 672-676.

Livak K.J. and Schmittgen T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25(4):402-8.

Maiuri M.C., Tasdemir E., Criollo A., Morselli E., Vicenci, J.M., Carnuccio R. and Kroemer G. (2009) Control of autophagy by oncogenes and tumor suppressor genes. Cell Death and Differentiation 16(1): 87-93.

Mashhadi Kholerdi A., Moradian F. and Mehralitabar H. (2024) In vitro study of the expression of autophagy genes ATG101, mTOR and AMPK in breast cancer with treatment of lactoferrin and in silico study of their communication networks and protein interactions. Progress in Biophysics and Molecuar Biology 190: 19-27.

Masini M., Bugliani M., Lupi R., del Guerra S., Boggi U., Filipponi F., Marselli L., Masiello P. and Marchetti P. (2009) Autophagy in human type 2 diabetes pancreatic beta cells. Diabetologia 52(6):1083-6.

Maycotte P. and Thorburn A. (2014) Targeting autophagy in breast cancer. World Journal of Clinical Oncology 5(3): 224.

Moradian F. and Mohammadzadeh S. (2023) Evaluation of the effect of nano-encapsulated lactoferrin on the expression of Bak and Bax genes in gastric cancer cell line AGS and study of the molecular docking of lactoferrin with these proteins. Gene 866:147355.

Moreno-Expósito L., Illescas-Montes R., Melguizo-Rodríguez L., Ruiz C., Ramos-Torrecillas J. and De Luna-Bertos E. (2018) Multifunctional capacity and therapeutic potential of lactoferrin. Life Sciences 195: 61-64.

Nurdinov N., Cinar V., Guler A., Yilmaz S.G., Kokcu N.D. and Hamurcu Z. (2021) LC3 and Beclin-1 as Markers of Autophagic Activity in Breast Cancer. Erciyes Medical Journal 43(4): 333-337.

Roshandel G., Ferlay J., Ghanbari-Motlagh A., Partovipour E., Salavati F., Aryan K., et al. (2021) Cancer in Iran 2008 to 2025: recent incidence trends and short-term predictions of the future burden. International Journal of Cancer 149(3): 594-605.

Russo M., and Russo G.L. (2018) Autophagy inducers in cancer. Biochemical Pharmacology 153: 51-61.

Safarian S. and Erfani A. (2021) Study of autophagy and cell death induction in MCF-7 cells in the presence of quercetin. Cellular and Molecular Research (Iranian Journal of Biology) 35(3): 1-11.

Shi Z., Li C.Y., Zhao S., Yu Y., An N., Liu Y.X. and Bao J.K. (2013) A systems biology analysis of autophagy in cancer therapy. Cancer Letters 337(2): 149-160.

Taji F., Mohseni Kouchesfehani H., Sheikholeslami F., Baesi K., Motavalli F. and Abdoli A. (2017) Induction of Autophagy by the Beclin 1 Gene and Its Effect on MDCK Cell Line Necrosis. Pathobiology Research 20(1): 1-15.

Tasdemir E., Maiuri M.C., Morselli E., Criollo A., D'Amelio M., Djavaheri-Mergny M., Cecconi F., Tavernarakis N., Kroemer G. (2008) A dual role of p53 in the control of autophagy. Autophagy. https://doi.org/10.4161/auto.6486

Watanabe T., Watanabe-Kominato K., Takahashi Y., Kojima M., Watanabe R. (2017) Adipose tissue-derived omentin-1 function and regulation. Comprehensive Physiology 7(3): 765–781.

Zendehdel K. (2020) Cancer Statistics in I.R. Iran in 2020. Basic and Clinical Cancer Research 12(4):159-165.

Zhang Y., Nicolau A., Lima C.F., Rodrigues L.R. (2014) Bovine lactoferrin induces cell cycle arrest and inhibits mTOR signalling in breast cancer cells. Nutrition and Cancer 66(8): 1371-1385.

Zhang Y., Lima C.F. and Rodrigues L.R. (2016) Anticancer effects of lactoferrin: underlying mechanisms and future trends in cancer therapy. Nutrure Review 72 (12): 763–773.