Identification of the effects of acid-resistant *Lactobacillus casei* metallopeptidase gene under colon-specific promoter on the colorectal and breast cancer cell lines

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**Abstract**

Objective(s): Anti-tumor effects of Lactobacilli as normal flora have been described. In a previous study, we identified a protein isolated from the bacterium *Lactobacillus casei* ATCC 39392 in acidic pH conditions named metallopeptidase. Therefore, we decided to evaluate the effect of the recombinant plasmid coding metallopeptidase protein on the inhibition, proliferation, or apoptosis of the colorectal and breast cancer cell lines.

Materials and Methods: Identified metallopeptidase gene of *L. casei* under the specific colon cancer promoter was transferred to the Human SW480 and MDA-MB231 cells. Cell viability was evaluated in these two cancer cell lines via MTT assay, apoptotic changes, and expression level of p53 and MAP2K1 genes in comparison with healthy blood cells as a control group.

Results: Viability of SW480 and MDA-MB231 cells was identified at 25% and 7%, respectively. An increase in apoptotic cell death in the SW480 cell line was observed as revealed by Tunnel staining. The expression assay of *TP53* and *MAP2K1* genes showed that MPL protein altered gene expression in a cell type-specific manner. Tunnel analyses showed that the pronounced cytotoxic effect of pEGFP-C2/MPL plasmid on SW480 cells was mediated through apoptosis.

Conclusion: These results suggest that endogenous recombinant MPL under colon specific promoter inhibits the proliferation of SW480 colorectal cancer cells by increase in MAP2K1 and P53 activation. *L. casei* metallopeptidase under the same circumstances could not affect the growth rate and viability of MDA-MB231 breast cancer cells in vitro.

**Introduction**

Colorectal cancer (CRC) is the third most common form of cancer (1). There is no specific way to identify the early stages of colon cancer, and advanced cancer treatment is by chemotherapy which has severe side effects and systemic toxicity because these drugs attack all dividing and replicating cells. Thus, there is a continuously growing demand for a novel anti-cancer agent (2). Breast cancer is another common malignancy, accounting for approximately one-third of all cancers occurring in women (3). According to the report of GLOBOCAN, the Global Cancer Observatory (2018), breast cancer incidence, mortality, and 5-year (2014–2018) prevalence were estimated to be the highest for women, whereas mortality and 5-year (2014–2018) prevalence for colorectal cancer incidence were ranked second and third for women and men, respectively (4). Therefore, various studies are being conducted to improve therapeutic approaches, including the development of effective biomarkers for CRC detection, the identification of biotherapeutic drugs with fewer side effects, including antibodies and therapeutic proteins and enzymes whose clinical effects have been proven in humans (5, 6). Considering the toxic side effects of chemotherapy in the treatment of cancer, anticancer drugs with minimal or no side effects of natural origin including probiotic *Lactobacillus* strains have recently received more attention.

The lactic acid bacteria (LAB), are a group of gram-positive, non-spore forming, fermentative, catalase-negative, non-motile microorganisms. Some LAB strains are well known for their probiotic properties (7). They are conventionally used as antimicrobial, anti-diabetic, immunomodulatory, bio preservative, immune system booster, and gut microflora (8). LAB may suppress the growth of bacteria that convert procarcinogens into carcinogens, thereby reducing the number of carcinogens in the human intestine. They also produce short-chain fatty acids in the colon, which acidify the environment. They can influence bacterial enzyme activity related to the production of carcinogenic compounds, such as beta-glucuronidase, nitroreductase, and azoreductase. Some of these beneficial effects may be attributed to secreted probiotic-derived factors, like soluble metabolites. Such metabolites have recently been identified as “postbiotic” mediators (9) which are secreted and released into the…

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environment by probiotic bacteria and participate in the interaction between symbiotic bacteria with the host epithelial and immune cells (10-12). According to Sanchez et al. findings, the commensal microbiota and the host cells have a continuous exchange of molecular information called cross-talk. Very little is known about the cellular receptors responsible for the recognition of extracellular proteins secreted by probiotic bacteria (11). Most of the characterized probiotic-host interactions take place through pattern recognition receptors (e.g., Toll-like receptors, TLRs) which recognize common molecules present on the bacterial surface (peptidoglycan and lipoteichoic acids) or derived from them (e.g., unmethylated CpG DNA and exopolysaccharides). After binding, components of probiotics like MAMPs to PRRs regulate nuclear factor kappa B (NF-kB), mitogen-activated protein kinases (MAPK), peroxisome proliferator-activated receptor gamma, and other signaling pathways (10,13). In addition, some metabolites produced by probiotics, such as secreted proteins (extracellular proteins), organic acids, indole, bacteriocins, extracellular vesicles, H2O2, and NO, protect the gut’s epithelial barrier by boosting mucus secretion by goblet cells, increasing the production of antimicrobial peptides, or enhancing the expression of tight junctions (14).

Probiotics change key signaling pathways in intestinal epithelial cells. Thereby the key biological signaling pathways like NFκB, MAPK, Akt/P13K, and PPARγ are targets for probiotics or their products (15). Previous studies have reported that different species of the genus Lactobacillus could inhibit colon cancer progression, however the exact molecules involved have not yet been identified (2). Lactobacillus casei ATCC 39392 is a key microorganism in fermented dairy products and foods. The anti-proliferative effects of this strain have been demonstrated on several cancer cell lines, including human (HT-29) and mouse (CT26) colon cancer cell lines (9). Supernatants and bacterial extracts of this standard bacterium were treated in CaCo-2 cells (16). The effect of growth inhibition of colon carcinoma in BALB/c mice with ATCC 39392 (2) and liver cancer Huh7 cells (17) have been reported. The beneficial effects of Lactobacillus secreted products that have been exerted through mechanisms similar to those described for probiotics include (1) competition with pathogens for binding to the receptors, nutrients, and colonization; (2) promotion of intestinal epithelial cell survival and barrier function; (3) stimulation of innate immunity and reduction of pathogen-induced inflammatory bowel diseases (18). Some of them are produced in the supernatant, while some are also structural components of the bacterium. The supernatants are not involved in the growth, proliferation, and evolution of bacteria, and are usually produced in the stationary phase of the growth of bacteria. Since there are a number of inherent problems in application of direct live bacteria, it seems using probiotic byproducts would be more beneficial and safer (19). Many studies on human mucosa organ culture have shown that some probiotics are detrimental to inflammatory bowel diseases. The study of extracellular proteins may provide novel strategies for clinical application of probiotic bacteria and may allow understanding of their mechanism of action and interaction between probiotic bacteria and the human host cells. Extracellular proteins secreted by probiotic lactobacilli have been shown to help maintain the mucosal barrier, mainly through MAPK-dependent mechanisms (11). Two protein produced by LGG, p40 and p75, have been shown to promote IEC homeostasis (6,8,12).

Based on Chull reports, a novel therapeutic probiotic-derived protein, P8, with anti-colorectal cancer (anti-CRC) properties in CRC cell line (DLD-1), had low penetrative efficiency, and they tried to improve delivery to CRC cells and expressed P8 as endogenous in order to improve P8 therapeutic efficacy, which doubled its anti-proliferative activity. They showed that endogenous P8 expression suppresses growth of CRC cells by inducing cell cycle arrest through inhibiting Cdki/Cyclin B1 activation via the p53-p21 pathway (5). Probiotics encounter extreme environmental conditions during food processing or along the gastrointestinal tract. In most cases, multiple biological functions are affected upon exposure of the cell to environmental stress. Sensing of sublethal environmental stress can allow for adaptation processes to occur, which can include alterations in the expression of specific proteins (20).

In some studies, the responses of various probiotics against different environmental stresses such as acid stress have been studied by comparative proteomic analysis to identify proteins important in acid tolerance and other environmental stress conditions (20-24). Similar to this type of research on therapeutic effects of this microbe, we examined the effects of acid stress on the intracellular proteome of L. casei ATCC 39392 and using proteomics methods we identified a metallopeptidase protein (25).

However, secretion and cell wall anchoring of proteins are key mechanisms of LAB interaction with their environment. Optimization of protein export is valuable when using these food-grade microorganisms for biotechnological applications both in vitro and in vivo in humans and animals, and for development of recombinant LAB as a live, vaccine-delivery vehicle (7).

In the present study, a gene putatively encoding a metallopeptidase protein of L. casei ATCC 39392 was cloned and characterized under specific colon cancer cell promoter. The anti-proliferative and apoptotic activities of the endogenously expressed metallopeptidase protein were evaluated on SW480 and MDA-MB231 cell lines and compared with WBC blood cells as control.

Materials and Methods

Metallopeptidase gene construction

The sequence of metallopeptidase protein conducted from mass spectroscopy of isolated protein in L. casei ATCC 39392 cultivation in acidic condition was consider for plasmid design. The peptide sequences of secreted protein were compared with protein sequences in the NCBI microbial blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlatSearch&BLAST_SPEC=MicrobialGenomes) using BLAST analysis. The sequence was retrieved from Uniprot (https://www.uniprot.org/). After reverse translation of protein sequence (https://web.expasy.org/translate/), it was codon-optimized for human cell culture by JCAT online server (http://www.jcat.de/) and named MPL. The colon cancer cell-specific promoter (GCTTCGCCCACACCTCCCTCCTCCCCTCGCGACGCCCAAGGCTTTGACGGCGCTATGGGCGCTTG-
GGATGGGCCCTGCTGCGGTGCTGGCTGGCTGGGCTGT- 
GGGGCCCGGGATCCGGCGGCGGCGGCGG
was obtained from 
the promoter database (https://epd.epfl.ch/index.php) 
which was inserted upstream of the metallopeptidase gene. This sequence was subsequently cloned into the pEFGFP-C2 eukaryotic expression vector between EcoRI and XhoI restriction sites with a hexahistidine tag at N-terminus and named pEFGFP-C2/MPL plasmid. The pEFGFP-C2 expression vector was used as a delivery vehicle for endogenous expression of MPL protein. Restriction 
stricton assay was applied for the confirmation of the synthesized plasmid.

**Cell lines, growth conditions, and transfection**

Human SW480 (ATCC® CCL-228™) and MDA-MB 231 (ATCC® HTB-26™) cell lines were purchased from the Pasteur Institute of Iran. They were cultured in standard DMEM (GIBCO, USA) medium supplemented with 10% fetal bovine serum (FBS) (GIBCO, USA), 2 mM glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin (GIBCO, USA) at 37 °C in a humidified atmosphere of 5% CO₂. The cells were washed twice with PBS, harvested with 0.05% trypsin, and transferred (10⁶ cells/ml) into a 24-well multi-dish (100 μl/well) containing serum-free fresh medium without antibiotics. The microplates were kept at 37 °C in 5% CO₂ until cell lines formed a monolayer in each well. We have used Lipofectamine® 3000 Reagent (Invitrogen) for transfection and delivered 10 μg plasmid per well containing SW480, MDA-MB 231, and blood WBC cell culture as control. The WBCs were collected using Ficoll® Paque Plus according to the manufacturer's instructions.

**GFP expression**

For evaluation of colon cancer specific promoter function, we inserted the MPL gene under promoter in upstream of the GFP gene in MPL construct. So, after 24 hr from transfection of all of the cells by pEFGFP-C2/MPL plasmid, they were studied by fluorescent invert microscopy (Juli, China) for GFP expression.

**Cell viability assays by micro culture tetrazolium test (MTT assay)**

Cell viability was determined using the MTT assay for transfected SW480 and MDA-MB 231 cells at an initial cell density of 10,000 cells per well. This experiment focused on analyzing the ability of expressed metallopeptidase gene derived from L. casei to inhibit colorectal cancer cells. 3-[(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), a colorless, transparent tetrazolium salt, is reduced to yield a purple formazan crystal through mitochondrial succinate dehydrogenase in living cells. In total, 100 μl (10⁵ cells/ml) of cells was seeded into a 96-well plate, and the cells were cultured overnight at 37 °C in a CO₂ incubator, which made the cells attach, divide and grow in the 96 wells. The culture medium was removed, 0.2 ml of plasmid and cell culture media without phenol red was added to the respective wells. The cells were incubated for 24 hr. For the analysis, 100 μl of MTT (5 mg/ml) solution was added to each well. After 2 hr culture at 37 °C in a CO₂ incubator, the supernatants were removed and 100 μl of dimethyl sulfoxide isopropanol was added to dissolve the purple formazan crystals. An ELISA reader (TECAN, Denmark) was used to read the absorbance at 570 nm; next, inhibitory rates were calculated to determine IC₅₀ values. The formula to calculate the inhibitory rate is as follows: Inhibition ratio (%) = [(OD control + OD treated) / (OD control)] × 100%.

**Determination of apoptosis by TUNEL test**

The apoptosis-inducing effect of metallopeptidase protein on SW480, MDA-MB 231, and blood WBC cells (10⁶ cells/well) in a 6-well plate was assayed using the TUNEL method with a commercial Dead-End Colorimetric TUNEL Detection Kit (Promega, UK) according to the manufacturer’s instructions. Imaging by light microscopy was used for studying and counting the cells.

**The expression analysis of P53 and MAP2K1 genes by real-time RT-PCR**

After 24 and 48 hr from plasmid delivery with three repeats, the cells were harvested and total RNA was extracted from the cells using the Gene All Hybrid-R RNA Purification Kit (Seoul, South Korea) according to the manufacturer's instructions. The quality and concentration of RNA were examined by ethidium bromide-stained agarose gel electrophoresis and spectrophotometric analysis. Complementary DNA (cDNA) template was synthesized from 1 μg RNA with 200 unit M-MuLv reverse transcriptase enzyme and 2 pmol oligo dT primer (Fermentas, Lithuania). The tubes were incubated at 25 °C for 10 min, 37 °C for 30 min, 42 °C for 60 min, and 70 °C for 5 min. Actin1 as the housekeeping gene and normalizer for all of the target genes (P53 and MAP2K1) was considered. PCR was used to confirm the quality of the synthesized cDNA. RT-PCR reactions were prepared in a final volume of 13 μl that contained 1 pmol of each primer (Table 1), 1 μg synthesized cDNA, 7 μl DNA Master SYBR Green I mix

**Table 1. Primers used in real-time RT-PCR of apoptosis pathway genes**

| Gene  | Primer sequence 5' - 3' | Fragment length (bp) |
|-------|------------------------|----------------------|
| TP53  | AGGACTAAGGGAGCACTG     | 215                  |
| TP53  | CTGGGAGTCCTTGAATGC   |                      |
| MAP2K1| GGGAGAAGACACTGGAGGCG | 250                  |
| MAP2K1| CATCCTGAGTTGTCCGCC   |                      |
| ACTN1 | TGTTGGACTGGATCTGGCAGCA | 200                  |
| ACTN1 | CATCCTGCCCTGAGGGGACATGAA |                  |

TP53: tumor protein p53; MAP2K1: mitogen-activated protein kinase; ACTN1: actinin alpha1; F: Forward; R: Reverse
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(Amligon, Denmark), and 4 µl nuclease-free water. Real-
time PCR was performed as follows: 95 °C for 5 min, 95
°C for 10 sec, 60 °C for 15 sec, 72 °C for 20 sec, and 40
cycles of extension. RT-PCR was carried out in a Step
One™ instrument (Applied Biosystems, Germany) with
actin 1 as the housekeeping gene.

Statistical evaluation
Statistical analysis of the study data was performed
using the graphpadprism 8 software package. One-way
analysis of variance (ANOVA) or independent sample
t-test was used to determine the statistical significance;
P<0.05 indicates significant difference.

Results
Confirmation of the MPL plasmid construct
The synthesized MPL gene downstream of the specific
colon cancer cell promoter (2271bp) was cloned into
a pEGFP-C2 vector (Figure 1). It was confirmed by
restriction analysis.

Colorectal cell-specific promoter performance
evaluation by GFP expression
Figure 2 shows GFP expression in the cancer cell lines,
24 hr after transfection, and confirms the promoter
efficiency that has been inserted before the GFP gene in
the plasmid structure (Figure 1). Thereby we expected
to express the MPL protein inside the cells.

Cytotoxic effects of MPL protein
With transfection of pEGFP-C2/MPL (MPL gene under
colorectal cancer promoter) into SW480 and MDA-MB231
cells, anti-proliferative effect of L. casei metallopeptidase
protein on cancer cell lines was assayed by cell viability
MTT kit. Figure 3 shows the proliferation of these cells
in the presence of the endogenous metallopeptidase
protein. At the concentration of 10 mg/ml of plasmid,
25% inhibition of SW480 cell proliferation was observed
at 48 hr of incubation. L. casei metallopeptidase has a
lower inhibition effect on the growth of breast cancer
cells (7%).
In SW480 negative cells (cells untreated with MPL)
the inhibition of growth and proliferation was 8%. In
SW480 positive cells (cells treated with MPL) the growth
inhibition was 25% in proportion to the control (SW480
cell line treated with pEGFP-C2 plasmid to evaluate the

![Figure 1. Schematic demonstration of pEGFP-C2/MPL plasmid. Metallopeptidase gene was cloned under the specific promoter in pEGFP-C2 (6984bp)](image1)

![Figure 2. Specific promoter confirmation. GFP expression in SW480 cells 24 hr after transfection with MPL plasmid](image2)

![Figure 3. Comparison of cell proliferation between transfected a) SW480 and b) MDA-MB 231 cells. (c) with vector plasmid, (-) without plasmid, (+) with plasmid](image3)
The results showed that MPL could inhibit the growth and proliferation of SW480 treated cells better than untreated and control cells but in the MDA-MB231 cell line, it was not significant. These results confirm the specific action of the used colorectal cell promoter.

**MPL plasmid promotes apoptotic cell death in colon and breast cancer cells but not in white blood cells as control**

Measuring the effects of this gene on cell death is important in studying the agent's mechanisms of action. Cell death was monitored by cell imaging using the TUNEL test. We observed signs of cell death in colon cancer cells incubated with the metallopeptidase gene. Delivery of 10 µg plasmid (pEGFP-C2/MPL) to SW480 and MDA-MB 231 cells for 24 hr showed a significant increase in the percentage of both early and late apoptotic cells as compared with healthy blood cells. The blood cells treated with this plasmid (Figure 4B) were healthy, characterized by a round-shape and unstained by TUNEL.

In the SW480 cell line (Figure 4C), a significant number of cells (about 90% of the cell population) stained dark brown and had undergone apoptosis. In MDA-MB 231 cells, the number of apoptotic cells was limited (20%) (Figure 4A).

**Metallopeptidase of Lactobacillus casei up-regulates expression of the TP53 and MAP2K1 genes in the apoptosis cascade**

In order to understand the molecular events leading to apoptosis by MPL expression, we examined the expression of two genes associated with apoptosis. The mRNA expression levels of TP53 and MAP2K1 in SW480 and MDA-MB 231 cell lines were measured by RT-qPCR. Following transfection, mRNA expression levels of TP53 and MAP2K1 were up-regulated compared with the control after 48 hr (Figure 5).

**Discussion**

All of the traditional cancer therapies, including various surgeries, hormonal therapies, immune therapies, etc., show a lack of efficacy in terms of the long-term outcome because of their failure to target cancer cells and toxicity due to non-specific effects on normal cells (26). Probiotics and gut commensals can modulate the host’s gut epithelial barrier function and offer therapeutic benefits (5). The present study evaluates the effects of an acid-resistant recombinant protein identified in the L. casei ATCC 39392 strain against growth inhibition of colon and breast cancers in a cell culture model.

In our previous study an ATP-dependent metallopeptidase (FtsH/Yme1/Tma family protein), from the lactic acid bacterium *L. casei* was identified by proteomics approaches (25). The metallopeptidase gene encodes a protein of 714 amino acid residues, which displays at least 90% identity with this protein in *Lactobacillus* genera. Its expression is induced after decreased pH from 7 to 5. This protein plays an important role in the physiology and survival of bacteria in intestinal acid conditions.

Thus, a gene fragment encoding metallopeptidase protein of *L. casei* ATCC 39392 under specific colon cancer cell promoter was considered and the anti-proliferative and apoptotic effects of the endogenous...
expressed metallopeptidase protein were evaluated on SW480 and MDA-MB231 cell lines and compared with WBC blood cells as control. Numerous studies have been done on the effect of this bacterium on cancers, including Tiptiri-Kourpeti’s research on the growth-inhibitory effects of L. casei ATCC 39392 against experimental colon cancer. He found administration of live L. casei (as well as bacterial components thereof) on murine (CT26) and human (HT29) colon carcinoma cell lines raised a significant concentration and time-dependent antiproliferative effect; live L. casei induced apoptotic cell death in both cell lines (9). Therefore, more detailed research on each of the proteins of this bacterium is necessary to obtain its effective protein and design drugs effective against cancer.

More work is needed in order to reveal the causative underlying characteristics responsible for specific antitumor effects. Survey effects of metallopeptidase isolated from Lactobacillus on SW480, MDA-MB231 showed that metallopeptidase induces p53 expression in SW480 cells and apoptosis occurs in the cells treated with this protein more than MDA-MB231 cells.

The application from tissue-specific promoters is one of the ways for cancer cells targeted therapy. According to Ghanbariasad et al. mammaglobin-1 expression is restricted to the mammary glands and no expression has been reported in various types of benign tissue or neoplasia other than breast carcinoma. They introduced the mammaglobin-1 promoter as a cancer specific promoter with high efficacy (31). Thus, colon cancer specific promoter may limit the damage to healthy cells by expressed MPL protein.

Also, for evaluation of the specificity of the promoter function, we study the cytotoxicity and growth inhibition of the endogenous metallopeptidase as a protein derived from Lactobacillus on human colon and breast cancer cell lines. MPL gene construct with specific promoter was found to inhibit the growth of colon cancer cells significantly as detected by the MTT assay. But it had no effect on the growth inhibition of the breast cancer cell line. This gene has the potential to inhibit the proliferation of SW480 cells.

In this regard, Kim et al. also investigated the effect of extracts of L. casei ATCC 39392 on human cervical cell lines Caski and HeLa, and asserted that the extract did not affect the growth of women’s cancer cell lines, and there was no synergistic effect after concomitant administration of one or more chemotherapeutic drugs. Thus, L. casei extract may have an anti-cancer effect in cervical cancer through an effect on cell cycle arrest, although it has no effect on growth inhibition or any synergistic effect on women’s cancer cell growth when administered together with a chemotherapeutic drug (32). Many studies have reported about the beneficial effects of metabolites of this genus of bacteria, for example, Chuah et al. showed that postbiotic metabolites (PM) produced by the six strains of L. plantarum exhibited selective cytotoxicity via antiproliferative effect and induction of apoptosis against malignant cancer cells in a strain-specific and cancer cell type-specific manner whilst sparing the normal cells (33). Thus, due to the differences between the two cancer cells in our study, the mechanisms and pathways of apoptosis induced under the treatment with the metallopeptidase encoded plasmid in the two cell types, did not demonstrate the same. Another research demonstrated that poly P, a cytoprotective compound from Lactobacillus brevis SBC8803 is the molecule responsible for maintaining intestinal barrier actions which are mediated through the intestinal integrin b1-p38 MAPK (34). These results indicate that metallopeptidase produced from the acidic metabolism in L. casei is most likely involved in the probiotic effects described for this bacterium. TP53 is a gene that interferes with cell cycle activities and is a tumor suppressor gene. When a tumor suppressor gene is mutated, cell proliferation is uncontrolled (35,36). Disruption of TP53 tumor suppressor gene regulation is one of the most common events in CRC stimulation, and gene reactivation may be a good suggestion for treatment of colon cancer. P53 can activate mitochondrial apoptosis pathways during cell stress. MAP2K1 is uniquely linked to TP53 mutation. In silico interaction between P53 and MAP2K1 has been shown (37). Therefore, a cell with a P53 mutated allele will lose its natural tendency to regulate programmed cell death and may survive due to genotoxic factors. Mutation in p53 is the most frequently detected genetic alteration in human cancers (38). Increased expression of this tumor suppressor protein leads to inhibition of tumor cell growth and apoptosis. Proteins isolated from probiotics lead to transcriptional activation of genes whose expression inhibits tumor growth. MAP2K1 is a member of the large family of Ser/Thr kinases, which triggers multiple rounds of hierarchical phosphorylation-activating kinase circles from the cell surface to the nucleus (39, 40-42). Also, mitogen-activated protein kinase (MAP kinase) is a key signal-transducing protein that transmits signals involved in both cell proliferation and apoptosis (43-45). In some articles have reported expression and clinical significance of MAPK in breast cancer (40, 46-49). Studies have examined the effect of different strains of Lactobacillus on breast cancer (50) and CRC (51).

Therefore, due to the important role of MAP2K1 and P53 proteins in preventing apoptosis, we decided to investigate the effect of endogenous protein expression of MPL with an intestinal cell specific promoter on expression of MAP2K1 and P53 in MPL protein treated human colorectal and breast cancer cell lines, and human normal white blood cells as a control. So, we proposed that this protein (metallopeptidase) inhibits cancer cell proliferation that is related to the modulation of apoptotic signaling-regulated proteins.

Conclusion
Transfer of plasmid-containing gene MPL under specific intestinal promoter by protein endogenous expression is able to induce apoptosis in colorectal cancer cells. This promoter acts specific and the MPL gene is not expressed in healthy and non-intestinal cells and does not cause harm.

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13099]. The authors declare no conflicts of interest.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

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