



## Different anti-inflammatory effects of *Lactobacillus acidophilus* and *Bifidobacterium bifiduum* in hepatocellular carcinoma cancer mouse through impact on microRNAs and their target genes

Zahra Heydari<sup>a</sup>, Mahdi Rahaie<sup>a,\*</sup>, Ali Mohammad Alizadeh<sup>b</sup>

<sup>a</sup> Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Postal Code: 1439957131, Tehran, Iran

<sup>b</sup> Cancer Research Centre, Tehran University of Medical Sciences, Postal Code: 1419733141, Tehran, Iran

### HIGHLIGHTS

- To find effects of the probiotics on liver carcinogenesis on gene transcripts.
- To find interaction microRNAs - target genes due to probiotics consumption.
- To find role of probiotics for removing destructive effects of carcinogen agent, AOM.

### ARTICLE INFO

#### Keywords:

Gene expression  
Cancer  
microRNA  
Probiotics  
*Bifidobacterium bifidum*  
*Lactobacillus acidophilus*

### ABSTRACT

Cancer is one of the most important causes of mortality in the world. General methods for cancer treatment have many side effects, while biological treatments such as probiotics consumption not only have no undesirable effects, but also are more acceptable method to treat the disease. Although probiotics have been recommended to therapy some diseases such inflammatory, infectious and neoplastic disorders, but their action mechanism is unknown. In this work, to investigate the inhibition effects of probiotics on Hepatocellular carcinoma and colorectal cancer progression, the genes involved in cancerous process were investigated in 38 Bulb/c mice. they divided into four groups including (I) Control (Healthy, without probiotic consumption) (II) Azoxymethane induced mice (III) AOM induced mice fed with *Lactobacillus acidophilus*, and (IV) AOM induced mice fed with *Bifidobacterium bifiduum* and the expression of four selected microRNAs and their target genes were analysed. The results showed that Azoxymethane, a potent colon carcinogen, treatment induced the expression of miR-221, miR-155 (in blood), Bcl-w and KRAS expression and decreased miR-122, PTEN and PU.1 expression in blood, but it has no effect on miR-18a in the liver tissue. The probiotic consumption enhanced miR-122 and PU.1 (in blood) as significant overexpression and down-regulated miR-221, miR-155 (in blood), Bcl-w and KRAS. Thus, the probiotics can help to control of cancer progression through postponing of metastasis process, reducing of inflammation and down and up-regulation of oncogenes/oncomirs and tumor suppressor genes/microRNAs, respectively.

### 1. Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent cancer in the world that exposes liver tissue and its molecular and biochemical mechanisms have been not good understood yet [1,2].

General treatment of cancer is based on surgery, radiation, chemotherapy and in current view, biological treatment. Biotherapy is a treatment that immune system against cancer is enhanced by biological agents. Use of probiotics is one of the biological treatments. Probiotic,

in term, is a food complementary includes of microorganism that it consuming by human, have useful effect on health due to desired change in gut microbial balance, hence it can be applied as biological treatment [3,4]. In fact, Probiotics are exterior live microorganisms which are consumed in the form of spores and in specific dosage and it believe that they are useful and have benefit for human health [5,6]. In the most of references, probiotic has defined as living microorganisms which by consumption of them and through change of the intestinal microflora balance, human health is conserved, positively [7]. It has

\* Corresponding author.

E-mail address: [mrahaie@ut.ac.ir](mailto:mrahaie@ut.ac.ir) (M. Rahaie).

<https://doi.org/10.1016/j.jnim.2019.100096>

Received 5 August 2018; Received in revised form 11 March 2019; Accepted 18 March 2019

Available online 20 March 2019

2352-3859/ © 2019 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

been found that probiotics have anticancer effects. The bacteria prevent colon from mutagenic and genotoxicity of chemical compounds and reduce the proliferation of cancer cells [4,8]. The production of anticarcinogenic compounds, promotion of immune system, the improvement of intestinal barrier, inhibition of cancer and cell proliferation, and apoptosis induction in cancerous cells are different kinds of probiotics actions. In human body, they must be able to tolerate the acidic and alkaline condition of stomach and gastric channel and survive in body. The consumption of health-promoting strains of microbe can help recovering the destructive interactions and hepatic disorders which it may arise as a result of interaction between bacterial components and hepatic receptors [9,10]. The most common used probiotics in industry include of lactobacillus, bifidobacterium and non-flammable yeast. Although the probiotics are recommended for application in inflammatory, infectious, neoplastic tumors and allergic disorders, but the list is not close and their number is increasing, continuously. Generally, Probiotics are analyzed for the variation of selected species, dose and quality of usage and their effectiveness and stability [11].

Several studies have shown that without any prior metabolism, colon epithelial cells can metabolize DMH (1,2-dimethylhydrazine) to carcinogenic metabolites. Furthermore, there is a general concept that the liver has a critical role in the DMH/AOM degradation *in vivo* and the resultant reactive intermediates are transferred to colon through blood or bile which in follow, carcinogenicity induction [12]. Azoxymethane (AOM) is a carcinogen for colon potentially which is metabolized by the liver to form genotoxic carcinogen methylazoxymethanol. AOM induces high levels of DNA damage in both colon and liver cells, however, tumours are formed almost exclusively in the colon [13]. The close relation between the liver and gastrointestinal tract with the role of liver as first receiver of the absorbed nutrients by the gut has caused to create a term named gut-liver axis.

Probiotics can also have a possible molecular mechanism via microRNAs (miRNAs) [14]. miRNAs as a new class of small noncoding RNA molecules are transcribed and processed in response to extracellular stimuli, or during the stages of development in a precisely regulated manner. They implicate in many cellular processes, including cardiovascular diseases [15], cancer [18] and they function as critical regulators of gene expression in multicellular eukaryotes and some unicellular eukaryotes [16,17]. In one of our published work, our results showed that the miRNAs could act as oncomiRs and tumour suppressor miRNAs after tumour growth and treatments [18]. In our previous study, the existence and stability of the probiotics were evaluated in gut microflora during five months after consumption of the probiotics [19]. It was also analysed several important immunity and tumour factors involved in colon cancer including, the T cells and the cytokines [20].

The aim of the study was investigation the effects of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* probiotics consumption on the expression of microRNAs including miR-122, miR-221, miR-18a and miR-155 and their target genes in liver tissue of AOM treated mice. The results can clearly be explained the molecular mechanisms of probiotics consumption effects on human cancer therapy.

## 2. Experimental

### 2.1. Materials

*L.acidophilus* (La5), source of CHR Hansen and *B.bifidum* (Bla/016P/M) with source of traditional product (yogurt) were obtained from Zist Takhmir Supplements Company (Tehran, Iran). Azoxymethane was provided from Sigma Aldrich Co. (St. Louis, USA). Total RNA extraction and Hybrid-R™ Blood RNA kits, Hybrid-R™ miRNA from Gene all<sup>R</sup> (Seoul, South Korea) were used for RNA and miRNA extraction. cDNA synthesis was done with Superscript III reverse transcriptase (Waltham, Massachusetts, Thermo scientific, USA) using oligo (dT) primers, Random Hexamer and specific primers (Macrogene, Seoul, South

Korea). Hot Firepol EvaGreen qPCR master mix was purchased from solis BioDyne Co. (Tartu, Estonia).

### 2.2. Animals

In this study, six weeks old Male Balb/c mice were purchased from Pasteur Institute of Iran (Tehran, Iran) and housed in plastic cages in room with controlled condition of 12 h light/dark cycle, humidity 50% and 23–25 °C temperature, and were given access to food and water ad libitum according to relevant national and international guidelines of the Weatherall report and Institutional Animal Care and Use Committee (IACUC) of Tehran University of Medical Sciences (Ethics Number 23797, In Tehran University of Medical Sciences, TUMS Institutional Review Board (IRB), which runs under the supervision of the Vice Chancellor for Research, is the core body responsible for this approval.).

### 2.3. Study design

The effect of *B.bifidum* and *L.acidophilus* probiotics was investigated on 38 mice to analyse microRNAs (miR-122, miR-221, and miR-18a, miR-155) and their target genes (Bcl-w, PTEN, KRAS and PU.1) expression in AOM-induced mice. The animals were divided into four groups including (I) control, common dietary food without any manipulation and probiotic consumption (healthy), (II) AOM-induced colon cancer mice (cancerous group, AOM group) (n = 10), (III) AOM induced mice fed with *L.acidophilus* (n = 9) ( $1 \times 10^9$  cfu/gr Bla/016P/M), and (IV) AOM induced mice fed with *B.bifidioum* (n = 9) ( $1 \times 10^9$  cfu/gr Bla/016P/M). To induce colorectal cancer, the mice were weekly (one injection per week) injected by AOM (15 mg/kg, s.c) for three continuous weeks and consumed common dietary food. For five months from ten days before AOM administration, Mice were fed with probiotics in group III and IV. During the period, all animals were monitored closely for general health. The animal were weekly weighed and investigated for evidence of rectal bleeding and death during the study. After five months, the mice were euthanized and their liver tissue were collected in RNase and DNase free microtubes and stored in –80 °C until more analysis.

### 2.4. Histopathological assay

10% formaldehyde was used to fix the colon tissues and then they passaged and embedded in paraffin. In follow, the Paraffin blocks were sectioned by 3 µm thickness for hematoxylin and eosin (H&E) staining. Slides were studied by OLYMPUS-BX51 microscope.

### 2.5. RNA extraction, cDNA synthesis and quantitative RT-PCR

MicroRNAs and total RNA from liver tissue and blood were isolated using the RNA and microRNA extraction kits (Hybrid-RTM miRNA and Hybrid-R™ Blood RNA, Gene all<sup>R</sup>, South Korea) according to provider's instruction with minor modification. The quality and quantity of extracted RNA were evaluated with a spectrophotometer (NanoDrop 2000C spectrophotometer, Thermo scientific, USA) and gel electrophoresis.

For primer designing, the sequence of the genes and microRNAs were derived from the gene bank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and miRbase ([www.miRbase.org](http://www.miRbase.org)), respectively. Primer 3 software was used to design PCR primers. mir-Q method was used to design microRNA primers for PCR reactions [21]. cDNA was synthesized with 200U of Superscript III reverse transcriptase (Thermo scientific, USA) using oligo (dT) primers, Random Hexamer for genes and microRNA specific primers. The expression levels of genes and microRNAs in the samples were measured by qPCR using Hot Firepol EvaGreen qPCR master mix (solis BioDyne, Estonia). qPCR was performed according to mir-Q method for microRNAs in Qiagen Real-time PCR System (Rotor-Gene Q, Germany) using primers of Table 1. The specificity of real-time PCR amplification was

**Table 1**  
The Primers used for mRNA and microRNA amplification.

microRNA/Gene	5'→3'
miR-122	F: 5-TGTCAGGCAACCGTATTCACCGTGAGTGGTCAAACA-3 R: 5-CGTCAGATGTCCGAGTAGAGGGGGAACGGCGTGGAGTGTGACAATGG-3
miR-221	F: TGTCAGGCAACCGTATTCACCGTGAGTGGTGA AAC R: CGTCAGATGTCCGAGTAGAGGGGGAACGGCGAGCTACATGTCTGTGCTG
miR-18a	F: TGTCAGGCAACCGTATTCACCGTGAGTGGTCAAGAAG R: CGTCAGATGTCCGAGTAGAGGGGGAACGGCGACTGCCCTAAGTGCTC
miR-155	F: TGTCAGGCAACCGTATTCACCGTGAGTGGTACCCCT R: CGTCAGATGTCCGAGTAGAGGGGGAACGGCGTAAATGCTAATTGTGAT
5s rRNA	F: GCCCGATCTCGTCTGATCT R: AGCCTACAGCACCCGGTATT
Universal primers	MP-fw: TGTCAGGCAACCGTATTCACC MP-rev: CGTCAGATGTCCGAGTAGAGG
Bcl-w	F: CTTTAGCAAACAGGAGCAGCAG R: AGACCAAGACCAACCCCTTAGC
PTEN	F: TACCTGGGCTCTGGACCATA R: TGCACAAACAAACAGCAGGAC
KRAS	F: ATCCCTGCTCTGTGCCATCTAC R: CAAAGGGAGCCTAAGTCTGTGAC
PU.1	F: AACAGATGCAGTCCTCGATAC R: ACAAGGTTTGATAAGGGGAAGCAC
β-actin	F: GGCTGGTATTCCTCCATCG R: CCAGTTGGTAACAATGCCATGT

confirmed by two criteria including a single band on agarose gel electrophoresis and a single peak in melting temperature curve of real time PCR-amplified products. GAPDH and β-actin genes were used as reference genes for normalization of data.

## 2.6. Statistics

Expression levels were calculated with the Ct values obtained from triplicate biological samples by Pfaffl method [22]. Their means were tested by LSD test for statistical significance with SPSS software ver. 21.0 (SPSS, Inc.) and  $P < 0.05$  was considered to be statistically significant.

## 3. Results and discussion

The major purpose of this study was to evaluate the effects of the *L. acidophilus* and *B. bifidum* probiotics consumption on Hepatocellular carcinoma, as a major liver cancer, protection in molecular level by expression analysis of miR-122, miR-221, miR-18a and miR-155, and their target genes, including Bcl-w, PTEN, KRAS and PU.1 in murine model. Fig. 1 shows a schematic of our methods and results in the work. The expression of miR-122-3p (less than half fold) and miR-18a-3p [23], as tumour suppressor microRNAs were down-regulated and non-significantly down-regulated, respectively, in liver tissue in AOM-treated group compared to control. But, the expression of miR-221, as an oncomir, was significantly up-regulated (5.5 fold). The oral consumption of these probiotics for five months, significantly increased, decreased and didn't affect the miR-122 (Fig.2a and 6), miR-221 (Fig.3a and 6) and miR-18a-3p (Fig.4a and 6) expression, respectively, in mice liver tissue compared to uneaten mice (AOM-treated or cancerous group).

It has been found the Bcl-w and KRAS genes act as oncogenes and PTEN acts as a tumour suppressor gene which their regulation is related to miR-122 [24], miR-18a and miR-22 [25], respectively. In our research, they (Bcl-w (Fig.2b and 6) and KRAS (Fig.4b and 6)) were up-regulated in liver tissue in AOM-treated animals more than 1.5 and 3 fold, respectively and PTEN was significantly down-regulated (0.4 fold, Fig.3b and 6), compared to control. The *L. acidophilus* and *B. bifidum* consumption decreased the expression level of the genes (Bcl-w and KRAS) significantly in mice liver tissue compared to uneaten mice (AOM-treated mice or cancerous group); however it couldn't significantly increase PTEN expression.

We showed that the expression of miR-122 and Bcl-w gene was decreased and increased in the cancerous group, respectively. MiR-122, a hepato-specific microRNA, is frequently down-regulated in human hepatocellular carcinoma. Moreover, Bcl-w is increased in hepatitis associated cirrhosis and probably it plays a role in hepatocarcinogenesis [24]. In present study, PTEN was down-regulated in cancerous group and therefore its expression due to decreasing of miR-221 expression should be increased by the probiotics consumption, however this elevation was not significant. PTEN is a potential target of miR-221, which is almost overexpressed in HCC [25]. PTEN is one of the most important tumour suppressors in human cancers and a key regulator of cell growth and apoptosis which is commonly altered [26]. In our work, we also showed that miR-18a and KRAS (Fig. 4a and b,6) were decreased and increased in the cancerous group (AOM treated), respectively (however the decrease of miR-18a was not significant); hence the consumption of probiotics bring back their expression. It has also been reported to target the KRAS gene by miR-18a and its functionality as a tumour suppressor and apoptosis, and proliferation [23,27]. To date, a few studies have shown that miR-18a specifically inhibits the KRAS expression in colon cancer cells [28]. MiR-18a may function as a potential tumour suppressor through targeting of KRAS [23]. MiR-18a targets specifically only on KRAS. *Ras* is the first human oncogene discovered by Shih et al. in the early of 1980s from a bladder cancer cell line [23].

In plasma, the expression of miR-155, as an oncomir, was up-regulated in AOM group compared to control (3.04 fold). The consumption of the probiotics for five months, decreased the expression level of miR-155 to less than one tenth in plasma compared to unconsumed group (AOM treated) (0.11 and 1.7 fold, respectively) (Fig.5a and 6).

PU.1 as a tumour suppressor gene related to miR-155 was down-regulated (0.32 fold) in cancerous group compared to control (Fig. 5b and 6). The probiotics increased PU.1 expression level in plasma in probiotic consumed groups (12.29 and 29.04 fold, respectively) (Fig. 5b and 6).

It has been shown that PU.1 mRNA is directly targeted by miR-155 in B cells [29,30]. PU.1 is a protein related to early B cell differentiation [29,31]. It is thought that the lack of PU.1 protein expression is associated with defective immunoglobulin transcription in HRS (Hodgkin and Reed/Sternberg) cells of cHL (classic Hodgkin Lymphoma) [32]. Micro-RNAs through pairing to the 3'-UTR of target mRNAs, are caused translational repression or sometimes, mRNA degradation [33]. Down expression of PU.1 due to miR-155 expression is critical for the

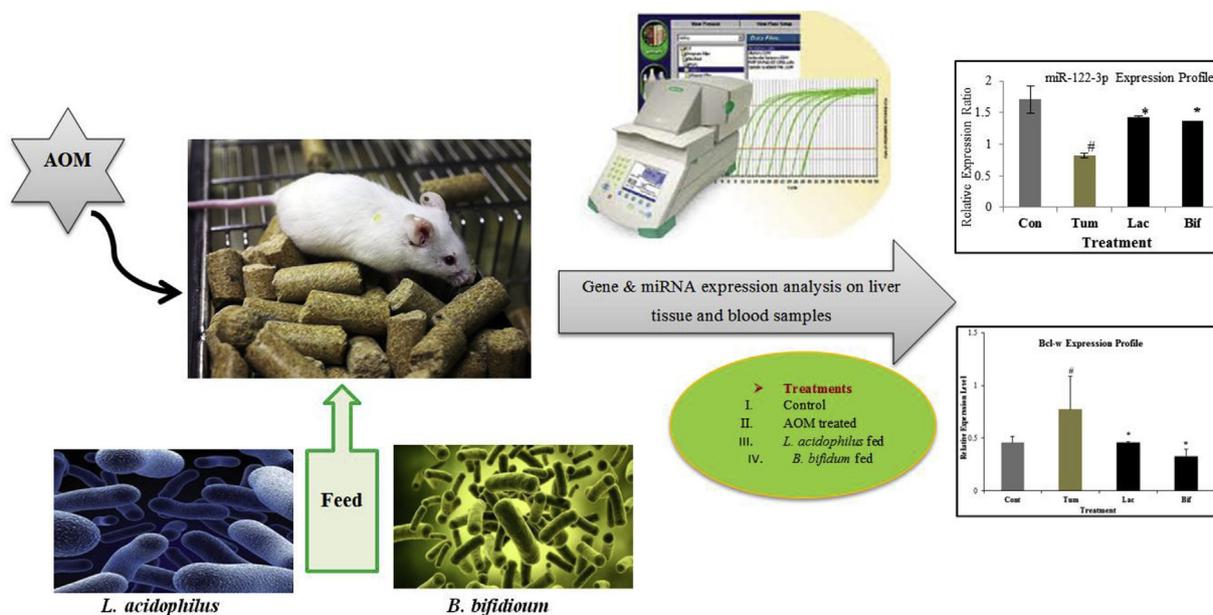


Fig. 1. A schematic of used methods and results of the work.

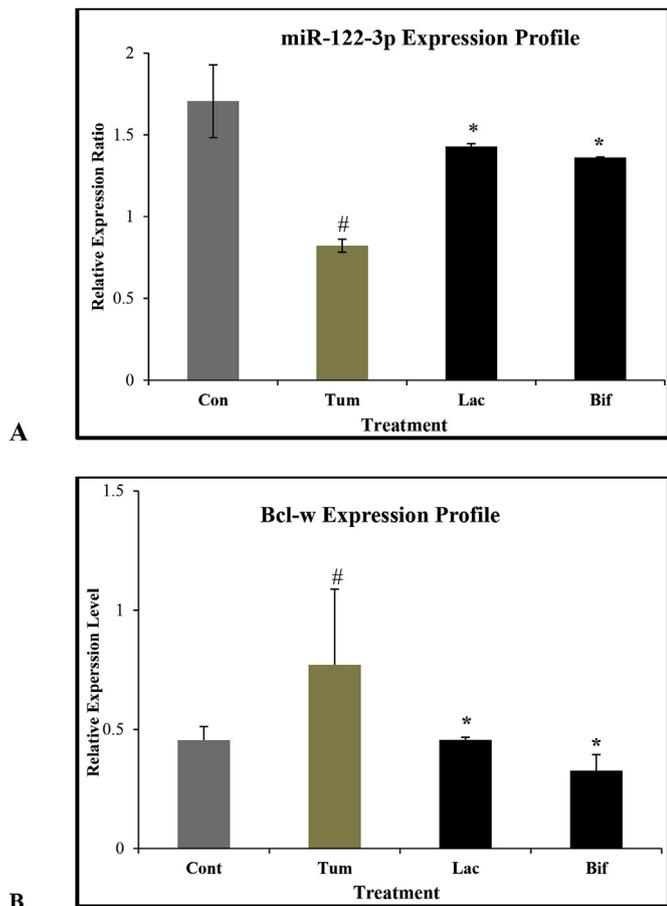


Fig. 2. The *Lactobacillus acidophilus* and *Bifidobacterium bifidum* consumption effect on (a) miR-122-3p and (b) Bcl-w expression in liver tissue. Data was presented as Mean  $\pm$  SD, \*P < 0.05 in comparison with AOM-treated group (Cancerous group) and #P < 0.05 in comparison with Control. Tum: AOM-treated, Lac: *Lactobacillus acidophilus* probiotic consumption, Bif: *Bifidobacterium bifidum* probiotic consumption.

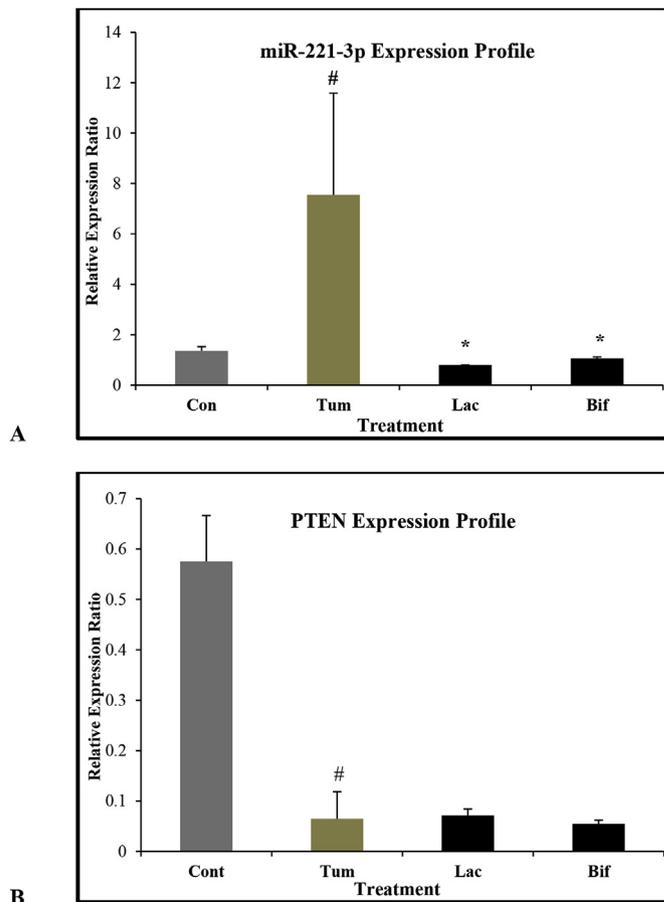
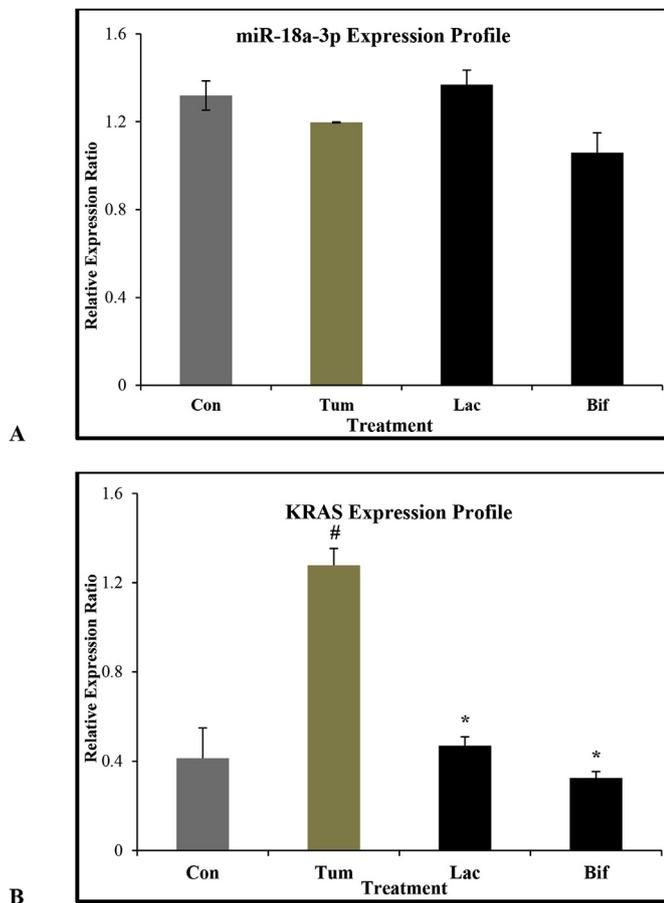


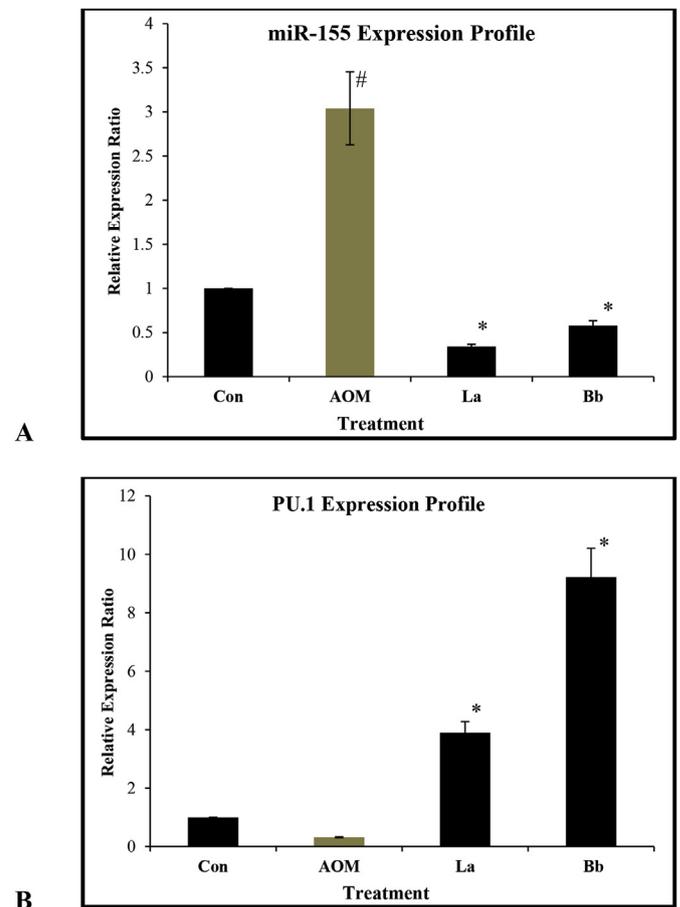
Fig. 3. The *Lactobacillus acidophilus* and *Bifidobacterium bifidum* consumption effect on (a) miR-221-3p and (b) PTEN expression in liver tissue. Data was presented as Mean  $\pm$  SD, \*P < 0.05 in comparison with AOM-treated group (Cancerous group) and #P < 0.05 in comparison to Control. Tum: AOM-treated, Lac: *Lactobacillus acidophilus* probiotic consumption, Bif: *Bifidobacterium bifidum* probiotic consumption.



**Fig. 4.** The *Lactobacillus acidophilus* and *Bifidobacterium bifidum* consumption effect on (a) miR-18a-3p and (b) KRAS expression in liver tissue. Data was presented as Mean  $\pm$  SD, <sup>\*</sup>P < 0.05 in comparison with AOM-treated group (Cancerous group) and <sup>#</sup>P < 0.05 in comparison to Control. Tum: AOM-treated, Lac: *Lactobacillus acidophilus* probiotic consumption, Bif: *Bifidobacterium bifidum* probiotic consumption.

production of IgG1 antibodies, properly [34]. In the other hand, miR-155 silencing in mouse is caused to defect in the B-cell humoral response to antigen due to impaired germinal centre formation [34,35]. Moreover, in PU.1 knock-out mice, the development of certain B-cell lineages is defected [35]. The IL-1 (Interleukin-1) pathway is controlled by miR-155 and it may regulate other inflammatory cytokine signalling cascades, but until now, its mode of action and the nature of its direct target(s) have been not found [29]. Our results showed that probiotics can decrease inflammatory by increasing PU.1 and decreasing miR-155 expression. According to above, the increase of miR-155 expression, activates the cascade of IL-1 and NF- $\kappa$ B (nuclear factor kappa B). Protective mechanism of probiotics was related to immunomodulation [36]. Therefore, it can be interpreted that probiotic feeding can help to decrease of inflammatory in colon tissue and protection of colorectal cancer.

In conclusion, it seems an ideal probiotic preparation would comprise of species with a human origin, since they are likely to be safe. Probiotics should be used only after enough clinical trials and clearing of their benefits. Also, the strain and dosage should be shown to be beneficial. Although probiotics are usually considered as safe but some complications have been reported. The cases of bacteremia, endocarditis, and fungemia have been observed, as well [37]. Nevertheless, our results showed the positive role of probiotic consumption during colorectal cancer and liver carcinogenesis in AOM induced cancer mice though the stimulation of expression of specific genes and miRNAs. Then, these are other documents for the usefulness of



**Fig. 5.** *L. acidophilus* and *B. bifidum* effects on (A) miR-155 and (B) PU.1 expression in plasma. Data was presented as Mean  $\pm$  SD, <sup>#</sup>P < 0.05 compared to control, <sup>\*</sup>P < 0.05 compared to AOM group (Cancerous group). Con: control (Healthy), AOM: azoxymethane treated, La: *Lactobacillus acidophilus* probiotic consumption, Bb: *Bifidobacterium bifidum* probiotic consumption.

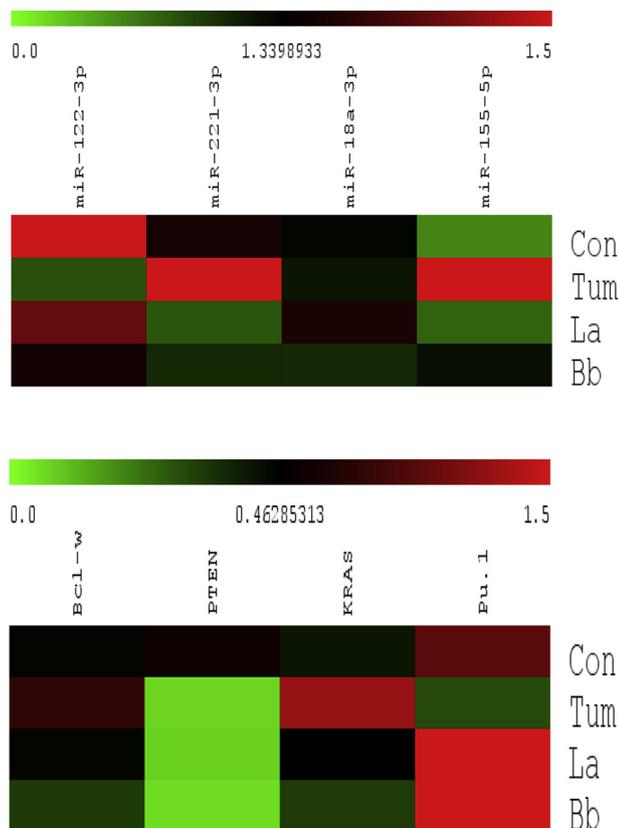
probiotics for health and protection against carcinogen agents. This study shows that the used probiotics may raise the sensitivity of cancer cells to treatment; even though it can't be recommended the probiotics consumption as a single cure way for cancer.

#### Funding

The financial supports provided by Iran National Science Foundation and University of Tehran and Cancer Research Centre of Tehran University of Medical Sciences.

#### Conflicts of interest

All authors declare that they have no conflict of interest.



**Fig. 6.** The heatmap of differentially expressed miRNAs and genes ( $P < 0.05$ ) in the Liver tissue and blood plasma. Con: control, TUM: azoxymethane treated, La: *Lactobacillus acidophilus* fed, Bb: *Bifidobacterium bifidum* fed.

### CRedit authorship contribution statement

**Zahra Heydari:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing - original draft. **Mahdi Rahaie:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Ali Mohammad Alizadeh:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision.

### Acknowledgment

We would to thank Iran National Science Foundation, University of Tehran and Cancer Research Centre of Tehran University of Medical Sciences for providing financial and instrumental supports in this work.

### Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jnim.2019.100096>.

### References

- H.B. El-Serag, K.L. Rudolph, Hepatocellular carcinoma: epidemiology and molecular carcinogenesis, *Gastroenterology* 132 (7) (2007) 2557–2576.
- K.S. Tummala, M. Brandt, A. Teijeiro, O. Grana, R.F. Schwabe, C. Perna, et al., Hepatocellular carcinomas originate predominantly from hepatocytes and benign lesions from hepatic progenitor cells, *Cell Rep.* 19 (3) (2017) 584–600.
- A.Q. Yu, L. Li, The Potential Role of probiotics in cancer prevention and treatment, *Nutr. Canc.* 68 (4) (2016) 535–544.
- W. Grajek, A. Olejnik, A. Sip, Probiotics, prebiotics and antioxidants as functional foods, *Acta Biochim. Pol.* 52 (3) (2005) 665–671.
- M.A. Ciorba, A gastroenterologist's guide to probiotics, *Clin. Gastroenterol. Hepatol.* 10 (9) (2012) 960–968.
- S. Bengmark, Bioecologic control of the gastrointestinal tract: the role of flora and supplemented probiotics and synbiotics, *Gastroenterol. Clin. N. Am.* 34 (3) (2005) 413–436 viii.
- M. Aghajanzpour, M.R. Nazer, Z. Obeidavi, M. Akbari, P. Ezati, N.M. Kor, Functional foods and their role in cancer prevention and health promotion: a comprehensive review, *Am J Cancer Res* 7 (4) (2017) 740–769.
- S. Dasari, C. Kathera, A. Janardhan, A. Praveen Kumar, B. Viswanath, Surfacing role of probiotics in cancer prophylaxis and therapy: a systematic review, *Clin. Nutr.* 36 (6) (2017) 1465–1472.
- V. Sharma, S. Garg, S. Aggarwal, Probiotics and liver disease, *Perm. J.* 17 (4) (2013) 62–67.
- S.A. dos Reis, L.L. da Conceição, N.P. Siqueira, D.D. Rosa, L.L. da Silva, MdCG. Peluzio, Review of the mechanisms of probiotic actions in the prevention of colorectal cancer, *Nutr. Res.* 37 (2017) 1–19.
- E.M. Quigley, Prebiotics and probiotics; modifying and mining the microbiota, *Pharmacol. Res.* 61 (3) (2010) 213–218.
- V. Megaraj, X. Ding, C. Fang, N. Kovalchuk, Y. Zhu, Q.Y. Zhang, Role of hepatic and intestinal p450 enzymes in the metabolic activation of the colon carcinogen azoxymethane in mice, *Chem. Res. Toxicol.* 27 (4) (2014) 656–662.
- A. Papanikolaou, R.C. Shank, D.A. Delker, A. Povey, D.P. Cooper, D.W. Rosenberg, Initial levels of azoxymethane-induced DNA methyl adducts are not predictive of tumor susceptibility in inbred mice, *Toxicol. Appl. Pharmacol.* 150 (1) (1998) 196–203.
- S. Kreuzer-Redmer, J.C. Bekurtz, D. Arends, R. Bortfeldt, B. Kutz-Lohroff, S. Sharbati, et al., Feeding of enterococcus faecium NCIMB 10415 leads to intestinal miRNA-423-5p-induced regulation of immune-relevant genes, *Appl. Environ. Microbiol.* 82 (8) (2016) 2263–2269.
- F. Pourrajab, F. Torkian Velashani, M. Khanaghaei, S. Hekmatimoghaddam, M. Rahaie, M.R. Zare-Khormizi, Comparison of miRNA signature versus conventional biomarkers before and after off-pump coronary artery bypass graft, *J. Pharm. Biomed. Anal.* 134 (2017) 11–17.
- M. Kato, F.J. Slack, microRNAs: small molecules with big roles - C. elegans to human cancer, *Biol. Cell* 100 (2) (2008) 71–81.
- C. Josse, N. Bouznad, P. Geurts, A. Irrthum, V.A. Huynh-Thu, L. Servais, et al., Identification of a microRNA landscape targeting the PI3K/Akt signaling pathway in inflammation-induced colorectal carcinogenesis, *Am. J. Physiol. Gastrointest. Liver Physiol.* 306 (3) (2014) G229–G243.
- S. Farsinejad, M. Rahaie, A.M. Alizadeh, M. Mir-Derikvand, Z. Gheisary, H. Nosrati, et al., Expression of the circulating and the tissue microRNAs after surgery, chemotherapy, and radiotherapy in mice mammary tumor, *Tumor Biol.* 37 (10) (2016) 14225–14234.
- H. Khavari-Daneshvar, M. Mosavi, H. Khodayari, E. Rahimi, P. Ranji, A.H. Mohseni, et al., Modifications of mice gut microflora following oral consumption of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* probiotics, *Turk. J. Med. Sci.* 47 (2) (2017) 689–694.
- S. Agah, A.M. Alizadeh, More Protection of *Lactobacillus Acidophilus* than *Bifidobacterium Bifidum* Probiotics on Azoxymethane-Induced Mouse Colon Cancer, (2018).
- S. Sharbati-Tehrani, B. Kutz-Lohroff, R. Bergbauer, J. Scholven, R. Einspanier, miR-Q: a novel quantitative RT-PCR approach for the expression profiling of small RNA molecules such as miRNAs in a complex sample, *BMC Mol. Biol.* 9 (2008) 34.
- M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, *Nucleic Acids Res.* 29 (9) (2001) e45.
- W.P. Tsang, T.T. Kwok, The miR-18a\* microRNA functions as a potential tumor suppressor by targeting on K-Ras, *Carcinogenesis* 30 (6) (2009) 953–959.
- C.J. Lin, H.Y. Gong, H.C. Tseng, W.L. Wang, J.L. Wu, miR-122 targets an anti-apoptotic gene, *Bcl-w*, in human hepatocellular carcinoma cell lines, *Biochem. Biophys. Res. Commun.* 375 (3) (2008) 315–320.
- M. Garofalo, G. Di Leva, G. Romano, G. Nuovo, S.S. Suh, A. Ngankee, et al., miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 down-regulation, *Cancer Cell* 16 (6) (2009) 498–509.
- A. Di Cristofano, P.P. Pandolfi, The multiple roles of PTEN in tumor suppression, *Cell* 100 (4) (2000) 387–390.
- K. Banno, I. Kisu, M. Yanokura, K. Tsuji, K. Masuda, A. Ueki, et al., Biomarkers in endometrial cancer: possible clinical applications (Review), *Oncol. Lett.* 3 (6) (2012) 1175–1180.
- M. Hiraki, J. Nishimura, H. Takahashi, X. Wu, Y. Takahashi, M. Miyo, et al., Concurrent targeting of KRAS and AKT by mir-4689 is a novel treatment against mutant KRAS colorectal cancer, *Mol. Ther. Nucleic Acids* 4 (2015) e231.
- E. Vigorito, K.L. Perks, C. Abreu-Goodger, S. Bunting, Z. Xiang, S. Kohlhaas, et al., microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells, *Immunity* 27 (6) (2007) 847–859.
- B. John, A.J. Enright, A. Aravin, T. Tuschl, C. Sander, D.S. Marks, Human microRNA targets, *PLoS Biol.* 2 (11) (2004) e363.
- J. Kluijver, S. Poppema, D. de Jong, T. Blokzijl, G. Harms, S. Jacobs, et al., BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas, *J. Pathol.* 207 (2) (2005) 243–249.
- R.T. Martinez-Nunez, F. Louafi, P.S. Friedmann, T. Sanchez-Elsner, MicroRNA-155 modulates the pathogen binding ability of dendritic cells (DCs) by down-regulation of DC-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN), *J. Biol. Chem.* 284 (24) (2009) 16334–16342.
- M. Chekulaeva, W. Filipowicz, Mechanisms of miRNA-mediated post-transcriptional regulation in animal cells, *Curr. Opin. Cell Biol.* 21 (3) (2009) 452–460.
- A. Rodriguez, E. Vigorito, S. Clare, M.V. Warren, P. Couttet, D.R. Soond, et al., Requirement of bic/microRNA-155 for normal immune function, *Science* 316 (5824) (2007) 608–611.
- T.H. Thai, D.P. Calado, S. Casola, K.M. Ansel, C. Xiao, Y. Xue, et al., Regulation of the germinal center response by microRNA-155, *Science* 316 (5824) (2007) 604–608.
- A. Sharma, B. Viswanath, Y.-S. Park, Role of probiotics in the management of lung cancer and related diseases: an update, *J. Funct. Foods* 40 (2018) 625–633.
- D.R. Snyderman, The safety of probiotics, *Clin. Infect. Dis.* 46 (Suppl 2) (2008) S104–S111 discussion S44–51.