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LncRNA HOTAIR Regulates the Function of the HMGCR Gene in Atorvastatin HepG2 Treated Cell

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ABSTRACT

Introduction: Statins are one of the approved drugs used in the clinic, which are prescribed to reduce the amount of cholesterol in the blood of patients. However, the effects of the drug in reducing the amount of fat and the occurrence of side effects are not the same in the patients. Due to the key role of LncRNAs in regulating gene expression, the possible role of HOTAIR LncRNA and atorvastatin treatment in regulating HMGCR gene expression as the main regulator in cholesterol synthesis has been investigated.

Methods: By the literature review, several LncRNAs that play a role in cell maintenance and homeostasis were identified. Bioinformatics analyses were used to find common regulatory factors between the *HMGCR* gene and candidate LncRNAs. MTT assay was used to determine the optimal dose of atorvastatin treatment in the HepG2 cell line. RNA extraction, cDNA synthesis, and quantitative analysis of gene expression were performed by qPCR. Finally, HMGCR protein expression was evaluated via the Western blot technique.

Results: Bioinformatic analyses showed that there is a relationship between HMGCR expression and some LncRNAs (*HOTAIR*, *TUG1*, *MALAT1*, *GAS5*, *JPX*, *DLX6AS*). In the cell culture, atorvastatin treatment increased the expression of HMGCR at mRNA and protein levels in the HepG2 cell line. Among the candidate lncRNAs, *HOTAIR* LncRNA expression decreased by 80% under atorvastatin treatment. Downregulating of the *HOTAIR* gene led to increased HMGCR expression at the RNA and protein levels.

Conclusion: The results of this study indicated that, aside from blocking the HMGCR enzyme binding site, atorvastatin can regulate the expression of HMGCR mRNA and protein by changing the *HOTAIR* expression.

Keywords: Cholesterol, Atorvastatin, HMGCR, LncRNA HOTAIR, Gene expression

INTRODUCTION

Cardiovascular diseases (CVD) are one of the main causes of death despite significant progress in prevention and treatment, and they impose substantial psychological, social, and economic pressure on society. Several risk factors have been identified that interfere with the initiation and acceleration of blood vessel occlusion. Managing these factors results in lowering the likelihood of developing cardiovascular

conditions. Among the risk factors, none has been as important as high cholesterol level (1). In the 90s, in a study called 4S, definitive evidence of the effect of cholesterol levels and its relationship with the risk of cardiovascular diseases was shown (2).

Cholesterol synthesis starts from acetyl-CoA through a series of reactions in the mevalonate pathway. This process mainly takes place in the liver and the conversion of acetyl-CoA to mevalonate is done by the rate-limiting enzyme called 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) (3). The first inhibitor of the HMGCR enzyme named Compactin was identified in 1973 (4). The continuation of the research led to the discovery of cholesterol-lowering drugs called statins, which act by competitively blocking the active site of the HMGCR enzyme, followed by reduced cholesterol synthesis. It increases the production of microsomal HMGCR and the LDL receptor expression on the cell surface, as a result of which the amount of LDL-C in the bloodstream decreases (5). Other researchers have shown that statins play a significant role in the prevention and management of CVD by reducing the level of LDL-C and C-reactive protein as a marker of inflammation (6). Statins can control the activity of the HMGCR enzyme through a negative feedback regulatory mechanism by reducing the level of intracellular sterol. In addition, the activity and amount of HMGCR enzyme are controlled at different levels such as post-translational modification, and destruction (7).

People with hypercholesterolemia do not respond equally to statin treatment due to genetic or non-genetic factors. It seems that the presence of different genetic variants such as single-nucleotide polymorphisms and haplotypes in regulators of cholesterol metabolism genes such as *HMGCR, APOE, PCSK9, ACE, LDLR,* and *ABC,* can play a role in the statin response (8).

Recently, it has been determined that long non-coding RNAs (LncRNAs) can regulate the function of various genes and influence growth, development, differentiation, and other biological processes. LncRNAs control gene expression through various mechanisms, including influencing mRNA stability and translation initiation, acting as a competitive internal RNA, and regulating post-translational modifications (9). On the other hand, along with the progress in sequencing technology, the importance of LncRNAs in a wide range of disease development and progression, inflammation processes, and drug responses is increasing (10).

Due to the importance of HMGCR function in statin consumption patients with hypercholesterolemia, in the present study, the effect of atorvastatin treatment on the expression of certain LncRNAs that are important in homeostasis and cell survival was investigated. In the following, since LncRNA HOTAIR showed the greatest change in expression by atorvastatin treatment in cell culture, we investigated the regulatory effect of LncRNA HOTAIR on the HMGCR expression, which is the main goal of statin for reducing cholesterol.

MATERIALS AND METHODS:

BIOINFORMATICS STUDIES

To investigate, candidate lncRNAs that are related to HMGCR expression, promoter, and regulatory factors such as transcription factors or microRNAs from the analysis of data available in miRNet databases (www.mirnet.ca), data ChipSeq and ENCODE were used.

Primer design

The sequences of all variants of the candidate genes were extracted from the NCBI site. The common sequences between the variants were identified by the Clustal Omega tool and primer design was done by Oligo 7 software. Finally, the specificity of the PCR product was ensured via NCBI-Blast. The sequences of primers and DNAzymes are given in Table 1.

Cell culture and cytotoxicity assay

The liver cancer cell line HepG2 was purchased from the Pasteur Institute of Iran. Cells were cultured in DMEM/F12 medium with 10% fetal calf serum (Gibco), 100 units of penicillin, and 100 micrograms/ml of streptomycin in 5% CO2 at 37°C. Atorvastatin was obtained from Dr. Abidi Pharmaceutical Company. For all experiments, atorvastatin was dissolved in 0.1% DMSO as a solvent.

Table 1. Sequence of primers and DNAzymes used in this research.

Gene	Sequence	NCBI ID	PCR product(bp)
GAPDH	F:5'-CCATGAGAAGTATGACAAC-3' R:5'-GAGTCCTTCCACGATACC-3'	NM_002046.7	115
HMGCR	F:5'-GTCACATGATTCACAACAGGTC-3' R:5'-TGCATGGAAAGAACCTGAGACC-3'	NM_000859.3	248
HOTAIR	F:5'-AGAAAAAGCAACCACGAAGC-3' R:5'-ACATAAACCTCTGTCTGTGAGTGCC-3'	NR_047517.1	167
TUG1	F:5'-GCTCTCTTTACTGAGGGTGCTT-3' R:5'-GGATCTGTCAAGTCTCAATGTTGG-3'	NR_152868.2	304
MALAT1	F:5'-CTAGCATCTTAGCGGAAGCTGA-3' R:5'-TATTGTGCTGTTACCTCCCACC-3'	NR_002819.4	305
GAS5	F:5'-CACAACAAGCAAGCATGCAG-3' R:5'-TCTTCTTGTGCCATGAGACTCC-3'	NR_152521.1	169
JPX	F:5'- GTAGACTGGGAGTGGAGGTTTG-3' R:5'- ACACTCTTTTCAGTGGCGGTTA -3'	NR_024582.1	182
DLX6-AS	F:5'- ATGATTCCTGTATGTATGGCAGCTA-3' R:5'- TGTTTAATCTGACCCTGCTGACA-3'	NR_015448.1	306
HOTAIR DNAzyme (12)	5'-TCGCTTTCAGGCTAGCTACAACGACTTCGTCTGG-3' 5'-CCTCATGGAAGGCTAGCTACAACGAACCAAATCAG-3'	ENSG00000228630	

To evaluate the drug toxicity, 5x103 cells were seeded in each well of a 96-well plate and the cells were treated with atorvastatin in different concentrations up to 160 µM for 24, 48, and 72 hours. After the end of each time, MTT solution was added to each well and finally, the absorbance of each well was read at 570 nm by a plate reader.

Atorvastatin treatment and HOTAIR DNAzyme transfection

Approximately 10^5 HepG2 cells were cultured in each well of a 12-well plate. Then for HOTAIR downregulation, we used HOATAIR-specific DNAzymes and Lipofectamin™ transfectant 2000 (Thermofisher Scientific; USA) According to the manufacturer's instructions. After 24 hours, atorvastatin treatment with 10 µM concentration was performed. The well-containing cells without DNAzyme and drug treatment were used as a control. Also, to ensure the correctness of the transfection, the vector encoding the GFP protein and the Scramble vector were used.

RNA extraction and cDNA synthesis

To assess the gene expression levels after cell treatment, RNA extraction was performed 24h after transfection using RNX Plus solution (SinaClon Company, Iran). The quality and quantity of RNA were determined by spectrophotometry or agarose gel electrophoresis. cDNA synthesis was performed with 3 micrograms of total RNA by an Easy cDNA synthesis kit (Parstuos Company, Iran). The PCR reaction was done with the GAPDH primers as an internal control gene to validate the cDNA production reaction.

Gene expression analysis by qPCR

The expression level of the HMGCR gene and target lncRNAs of *HOTAIR, TUG1, MALAT1, GAS5, JPX, and DLX6-AS* were evaluated by the qPCR technique. The relative expression of mRNA samples was normalized to the *GAPDH* gene. The PCR reaction was performed using 10 ng of cDNA, 2 µl of Eva Green (5X, Solis BioDyne, Estonia), 4 picomoles of each of the forward and reverse primers, and qPCR was performed to a final volume of 10 µl. The gene expression analysis and fold change value were done using $2^{-\Delta CT}$ and $2^{-\Delta\Delta CT}$ methods, respectively. All experiments were performed at least in duplicate.

Western blot

To evaluate HMGCR protein expression, total protein was extracted from HepG2 cells using a lysis buffer (RIPA) containing protease and phosphatase inhibitor cocktail. The extracted protein concentration was measured using the Bradford test at a wavelength of 490 nm. 20 µg of total proteins were electrophoresed on 10% polyacrylamide gel. Then, the products were transferred to PVDF membranes and 5% BSA solution was used to block the membrane. Incubation with the primary antibody was done at 4°C overnight, and then the membranes were incubated with secondary antibodies bound to HRP. Specific protein bands were evaluated by a luminescence kit (ECL, Amersham, England). GAPDH protein was used as an internal control. Finally, all the bands were quantified using ImageJ software.

Statistical analysis

Drawing graphs and statistical analyses were done using GraphPad Prism (version 9) software. Unpaired t-tests were used in 2 groups and more than 2 groups were analyzed by the Ordinary one-way ANOVA. The results for each group are shown with mean and standard deviation (Mean ± SD) and P-value ≤ 0.05 was considered as a significance level.

Table 2: Common micro-RNA and transcription factor between HMGCR and candidate lncRNAs

lncRNA	Common micro-RNA	Common TF
HOTAIR	hsa-mir-148b-3p, hsa-mir-19a-3p, hsa-mir-19b-3p, hsa-mir-130a-3p, hsa-mir-1-3p	BRCA1, CEBPB, CHD1, CHD2, ZNF384, USF2, CTCF, ZKSCAN1, TCF7L2, TRIM28, EP300, EZH2, FOXA1, GATA3, GTF2F1, H2AFZ, HDAC2, HDAC6, KDM5A, KDM5B, MAFK, MAX, MAZ, MXI1, POLR2A, TBP, RAD21, RBBP5, RCOR1, RFX5, SUZ12, SRF, STAT3, SMC3…
MALAT1	hsa-mir-32-5p,hsa-mir-92a-3p, hsa-mir-329-3p, hsa-mir-92b-3p, hsa-mir-362-3p, hsa-mir-181a-5p, hsa-mir-181b-5p, hsa-mir-181c-5p, hsa-mir-181d-5p, hsa-mir-185-5p, hsa-mir-216b-5p, hsa-mir-25-3p, hsa-mir-3140-3p, hsa-mir-3179, hsa-mir-338-3p, hsa-mir-582-5p, hsa-mir-205-5p, hsa-mir-101-3p, hsa-mir-1-3p, hsa-mir-23b-3p, hsa-mir-23a-3p, hsa-mir-942-5p	ARID3A, ATF1, ATF2, BACH1, BCL3 BCLAF1, BHLHE40, BRSA1, CBX3, CCNT2, CEBPB, CEBPD, CHD1, CHD2, CHD7, CREB1, CTCF, CUX1, E2F4, E2F6, EBF1, EGR1, ELF1, ELK1, EP300, ETS1, EZH2….
TUG1	hsa-mir-27a-3p, hsa-mir-29a-3p, hsa-mir-29b-3p, hsa-mir-29c-3p, hsa-mir-335-5p, hsa-mir-1276, hsa-mir-132-3p, hsa-mir-132-3p, hsa-mir-15a-5p, hsa-mir-15b-5p, hsa-mir-16-5p, hsa-mir-185-5p, hsa-mir-186-5p, hsa-mir-196a-5p, hsa-mir-212-3p, hsa-mir-27b-3p, hsa-mir-3179, hsa-mir-361-5p, hsa-mir-497-5p, hsa-mir-582-5p,hsa-mir-9-5p, hsa-mir-128-3p	ARID3A, ATF2, BACH1, BCLAF1,BHLHE40, BRCA1, CCNT2, CEBPB, CEBPD, CEBPZ, CHD1, CHD2, CREB1, CTCF, CUX1, E2F4, E2F6, EBF1, EGR1, EP300, ELF1, ELK1, FOXM1, FOXP2, GABPA, GATA1, GATA2…
GAS5	hsa-mir-29a-3p, hsa-mir-205-5p, hsa-mir-23b-3p, hsa-mir-23a-3p, hsa-mir-128-3p, hsa-mir-582-5p, hsa-mir-361-5p, hsa-mir-196a-5p, hsa-mir-335-5p, hsa-mir-29c-3p, hsa-mir-29b-3p, hsa-mir-335-5p	ARID3,AATF1, ATF2, BACH1, BCL3, BCLAF1, BHLHE40, BRCA1, CBX3, CCNT2, CEBPB, CEBPD, CHD1, CHD2, CREB1, CREB1, CTCF, CUX1, EP300, E2F4, E2F6, EBF1, EGR1, ELF1, ELK1, ETS1, EZH2, FOS, FOSL2, FOXA1, FOXA2, FOXM1, FOXP2, GABPA, GATA1, GTF2...
JPX	hsa-mir-32-5p, hsa-mir-92a-3p, hsa-mir-193b-3p, hsa-mir-92b-3p, hsa-mir-25-3p, hsa-mir-33a-5p, hsa-mir-33b-5p, hsa-mir-19a-3p, hsa-mir-19b-3p	ARID3A, ATF2, ATF3, BCL3, BCLAF1, BHLHE40, BRCA1, CTBL1XR1BX3,CCNT2, CEBPB CEBPD, CHD1, CHD2, CHD7, CREB1, CTCF, E2F4, E2F6, EBF1,EGR1, ELF1, ELK1, EP300, ETS1, EZH2, FOS, FOSL2, FOXA1, FOXA2, FOXM1,FOXP2, GABPA, GATA1, GATA2, GATA3, GTF2B, GTF2F1, H2AFZ, HCFC1, HDAC1, HDAC2, HDAC….
DLX6-AS	hsa-mir-1276, hsa-mir-15a-5p, hsa-mir-15b-5p, hsa-mir-16-5p, hsa-mir-497-5p	CTCF, POLR2A, SP1, STAT3, TAF1, EZH2, REST, TCF12, BACH1

RESULTS

Bioinformatics studies

Bioinformatics studies showed that 6 LncRNAs (*HOTAIR*, *MALAT1*, *TUG1*, *GAS5*, *JPX*, and DLX6-AS) have a common binding site for some miRNAs with *HMGCR* mRNA. Also, common transcription factors were found for the promoter of some of these LncRNAs with the *HMGCR* gene promoter. Analysis of metabolic networks showed that some of these transcription factors are involved in cholesterol biosynthesis pathways. The results are given in Table 2.

Finding the optimal atorvastatin concentration

MTT assay was used to select the appropriate concentration for atorvastatin treatment in the HepG2 cell line. Our results showed that the cell viability was reduced depending on the concentration and time of treatment. Finally, to investigate the physiological function of atorvastatin, a concentration of 10 µM and a 24-hour treatment were selected for all next experiments (Figure 1).

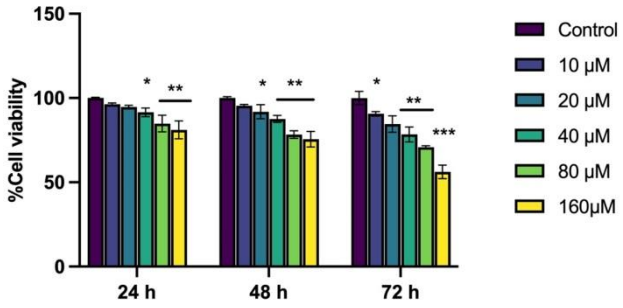

Figure 1: The effect of atorvastatin treatment on the viability of the HepG2 cell line. To find the optimal concentration of atorvastatin, the treatment was performed at different concentrations and times. Based on obtained result the concentration of 10 µM had no significant effect on the survival of HepG2 cells in 24 and 48 h.

HMGCR expression increased in response to atorvastatin treatment

To evaluate, the effect of selected drug concentration, HMGCR expression was analyzed in the HepG2 cell line in control and atorvastatin-treated conditions. The expression of the *HMGCR* gene (P-value=0.04, fold change=2.22) was increased in response to atorvastatin treatment (Figure 2).

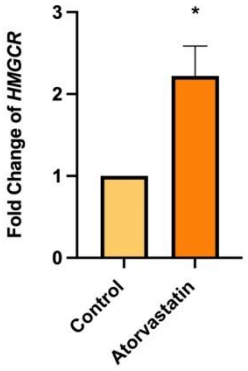

Figure 2: Atorvastatin treatment changes the *HMGCR* expression compared to control. The results of gene expression analysis showed that *HMGCR* expression increased (fold change = 2.22) in treatment with atorvastatin. Treatment was done at 10 µM for 24 h.

Atorvastatin treatment affects the expression of some candidate lncRNAs

The existence of common regulatory elements between the *HMGCR* gene and the candidate lncRNA promoters showed that each of the candidate lncRNAs may play a role in the response to statin and the regulation of *HMGCR* gene expression. To investigate this hypothesis, the analysis of the expression of these LncRNAs in HepG2 cells was performed in the presence and absence of the drug. Gene expression analysis showed that the expression of the *HOTAIR* gene (P-value = 0.004, fold change = 0.22), and *TUG1* (P-value = 0.84, fold change = 0.48), were decreased after atorvastatin treatment, in contrast, the expression of *MALAT1* (P-value = 0.07, fold change =1.6), *GAS5* (P-value = 0.11, fold change = 1.51), *JPX* (P-value = 0.23, fold change = 1.29), and *DLX6*-AS (P-value = 0.1, fold change = 1.54) were increased. Finally, based on the obtained result, the lncRNA *HOTAIR* was selected for further study (Figure 3).

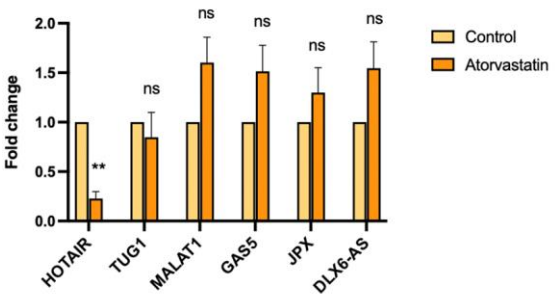

Figure 3: The expression of candidate LncRNAs after atorvastatin treatment compared to the control. The results showed that the expression of HOTAIR, and TUG1 genes decreased while the expression of MALAT1, GAS5, JPX, and DLX6-AS, genes increased. The most substantial alterations were noted in the expression levels of HOTAIR (P-value = 0.004). Treatment was done at 10 μM for 24 h.

Knocked-down of *HOTAIR* LncRNA expression

To evaluate, the possibility of a relationship between the *HMGCR* gene and *HOTAIR* lncRNA expression, gene expression was knocked down by a specific DNAzyme (11). *HOTAIR* expression after transfection of specific DNAzyme was investigated by qPCR. The DNAzyme was able to reduce the *HOTAIR* expression (P-value=0.008, Fold change=0.24) compared to the HOTAIR expression in the control cell (Figure 4a). Then, the effect of atorvastatin treatment or in combination with HOTAIR DNAzyme treatment on *HMGCR* expression was investigated. Atorvastatin treatment decreased *HOTAIR* expression in HepG2 cells (Figure 4-a). Whereas HOTAIR downregulation led to increased HMGCR gene expression (P-value=0.57 Fold change=1.92), which is similar to the effect of atorvastatin treatment on HepG2 cells. However, HOTAIR knockdown and simultaneous treatment with atorvastatin for 24h, showed a synergistic effect on HMGCR expression (P-value=0.07 Fold change=3.44) (Figure 4b).

Knocked-down of *HOTAIR* and atorvastatin treatment increased HMGCR protein

To investigate the effect of drug treatment or *HOTAIR* downregulation on HMGCR protein levels, the western blot assay was performed in HepG2 cells. Our results showed that the expression of HMGCR protein was increased (fold change=1.34) and (fold change=1.36) under atorvastatin treatment and downregulation of *HOTAIR*, respectively. Also, reducing the expression of *HOTAIR* before treatment with atorvastatin had a similar effect on the expression of HMGCR protein and led to increased HMGCR expression (fold change = 1.27). Our results showed the similarity between the results obtained from gene expression analysis at the protein and mRNA levels (Figure 4C and D).

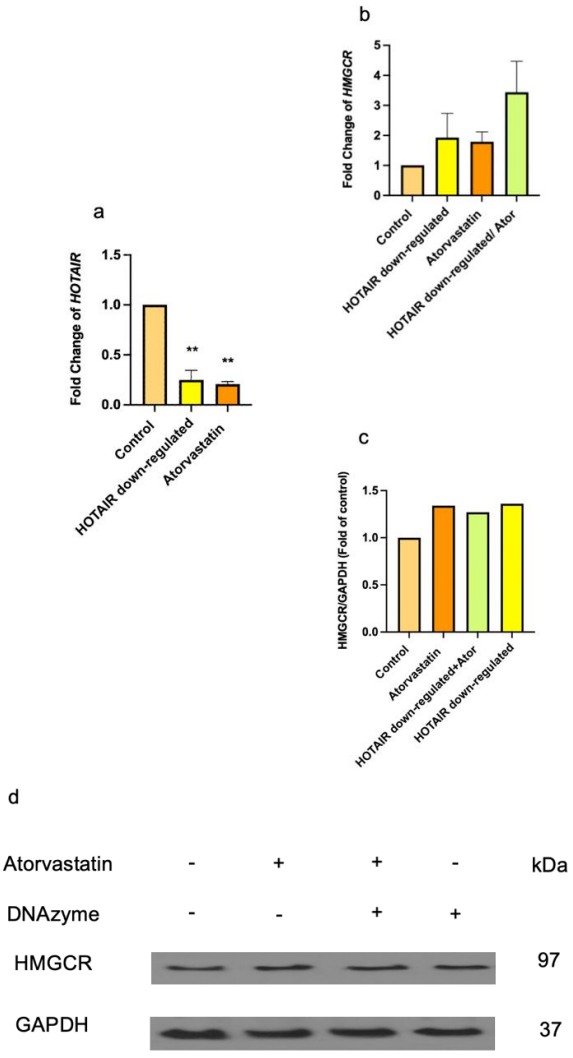

Figure 4: LncRNA HOTAIR controls HMGCR expression. a) The effect of knockdown of HOTAIR and atorvastatin treatment on HOTAIR expression. b) The effect of reducing LncRNA HOTAIR expression and atorvastatin treatment on HMGCR expression. Simultaneous treatment of HepG2 cells with atorvastatin and HOTAIR knockdown increased the HMGCR expression more than three times compared to the control cells. c and d) The effect of reducing HOTAIR expression and atorvastatin treatment on HMGCR protein expression. The results of western blot analysis showed that HMGCR protein expression increased as a result of HOTAIR downregulation as well as atorvastatin treatment.

DISCUSSION:

Cardiovascular diseases are the main cause of death worldwide and their prevalence has increased greatly in recent decades (12). The first direct association between blood cholesterol levels and CVD was identified by the Framingham Heart Study (FHS) and the Multiple Risk Factor Intervention Trial (MRFIT) (13). Therefore, lipid profile management is of special importance to prevent CVD.

Statins, cholesterol-lowering drugs, are the first treatment to prevent the occurrence of cardiovascular diseases. Independently of their fat-reducing properties, statins have pleiotropic effects on diabetes, neurological diseases, coronary heart disease, inflammation, and cancer (14). The known way of action of statins is inhibiting the HMGCR, the limiting enzyme of the cholesterol biosynthesis pathway, but individuals provide distinctive answers in treatment with these group of drugs (15). Previous studies showed that the presence of different genetic variants and alternative splicing in the HMGCR gene causes a variable response in cholesterol and LDL levels. (8).

However, all the pathways affected by statins are unknown. One of the current important issues is finding out how HMGCR or cholesterol biosynthesis pathways are affected by the use of atorvastatin. Previous research has shown hepatotoxicity in mouse models with HMGCR deletion. This study showed that endoplasmic reticulum stress followed by apoptosis was observed in the liver cells of mice with HMGCR deletion (16). This behavior cannot be related only to the inhibitory action of the HMGCR.

At present, the role of lncRNA in gene expression regulation and their participation in the creation or the progress of a wide range of diseases and drug metabolism is becoming clear (17-19). Therefore, we use bioinformatics tools to find other possible targets of atorvastatin on gene regulatory mechanisms. Using the *HMGCR* gene sequence, miRNAs that can bind to mRNA or its promoter were identified. Then comparing these factors with the sequence of some LncRNAs, showed that 6 LncRNAs (*HOTAIR*, *TUG1*, *MALAT1*, *GAS5*, *JPX*, and *DLX6*-AS) have common regulatory elements with *HMGCR*.

The effect of atorvastatin treatment on the expression of the mentioned candidate lncRNAs and its impact on the expression of HMGCR was investigated in HepG2 cells. These LncRNAs can play a role in cell maintenance. (20-25). Based on the obtained expression results, all candidate LncRNAs were affected by atorvastatin treatment. Meanwhile, *HOTAIR* showed the highest significant expression changes. Bioinformatics analysis showed that there are common transcription factors regulating the expression of the HMGCR and HOTAIR promoters, among which NRF1, GATA2, EP300, and SP1 are the most significant. These transcription activators have roles in lipid biosynthesis pathways. In addition, there are common miRNAs between HMGCR and HOTAIR (Table 2). Recently, HOTAIR's importance in lipid metabolism pathways has been mentioned. The study showed that HOTAIR expression was induced during adipose tissue differentiation (26). In addition, another study showed that HOTAIR downregulation reduced lipid accumulation through the miR-130b-3p/ROCK1 axis in the HepG2 cells (27).

Our data showed that atorvastatin increased HMGCR expression at the RNA and protein levels in HepG2 cells. The review of the articles showed that in the study of Luo et al., the use of statin led to an increase in the expression of the HMGCR in HepG2 cells, which was in line with the present results (28). In addition, it has been shown that treatment of male C57BL/6J mice with lovastatin increased HMGCR expression at both mRNA and protein levels in the liver of mice. Also, this group showed that treatment with an HMGCR inhibitor can increase the effectiveness of statin and reduce atherosclerotic plaques (29).

Our results showed that the *HOTAIR* expression decreased by atorvastatin treatment, this decrease in expression can be the result of the presence of estrogen response elements in the HOTAIR promoter (30). According to previous studies, statin leads to a decrease in cholesterol production, followed by decreasing in estrogen hormone production. Therefore, it seems that treatment with atorvastatin can lead to a decrease in *HOTAIR* expression. Then, to investigate the possible role of *HOTAIR* in HMGCR regulation,

the expression of the *HOTAIR* was reduced via the specific DNAzyme. Our data showed that HMGCR expression increased at the RNA and protein levels after the *HOTAIR* downregulation, and this decrease led to an increase in the atorvastatin effect on HMGCR expression. It seems that the cell has compensated HMGCR inhibition due to needing its function in the cholesterol biosynthesis pathway using atorvastatin treatment because atorvastatin increases the HMGCR transcription through a regulatory mechanism. The presence of miRNAs that interact with HMGCR and LncRNA HOTAIR gene transcripts may account for the increased expression of the target gene. This phenomenon can be explained by the sponging effect occurring at both transcriptional and translational levels.

According to the present results, the study by J-L Pang et al. showed that the expression of *HOTAIR* decreased in the blood of people with atherosclerosis. Also, in the cell model induced by ox-LDL, it was found that increasing the expression of *HOTAIR* led to a decrease in the accumulation of lipids in the cell (31). In our study, *HOTAIR* downregulation led to increased HMGCR expression at the level of RNA and protein. It seems that the increase in the expression of HMGCR can be caused by the epigenetic effect of *HOTAIR* on the promoter function of HMGCR. Previous studies have shown that LncRNA *HOTAIR* can lead to changes in the function of genes by histone modification, chromatin reconstruction, and assembly of PRC2 and LSD1 complexes on the regulatory region of the target gene (32). Therefore, it seems that *HOTAIR* lncRNA can affect the regulation of HMGCR expression followed by the cholesterol metabolism regulation in the cell. The present study is the first to demonstrate the regulation mechanism of HMGCR by *HOTAIR* lncRNA under statin treatment conditions. Previously, there have been studies related to the regulatory mechanisms of LncRNAs on HMGCR, for example, it has been stated that *ZFAS1*LncRNA increased HMGCR mRNA stability through U2NF2 and plays a role in controlling lipid metabolism in cancer cells (33). It shows the necessity of investigating several regulatory axes through which HOTAIR can change HMGCR expression and affect statin effectiveness.

CONCLUSION

The findings of the present study indicated that, in addition to the previously proven inhibitory effects of atorvastatin on HMGCR enzyme activity, atorvastatin can regulate its expression at both the mRNA and protein levels by altering the expression of certain lncRNAs. Also, the presence of genetic variants can affect the function of LncRNA HOTAIR, its resulting genetic and epigenetic regulatory mechanisms may affect the response rate of individuals when taking atorvastatin to reduce the risk of heart disease.

FUNDING

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Authors' Contributions

Sh. J. conducted the experiments and wrote the initial draft of the manuscript. S.Z. A. contributed to the bioinformatics analyses and interpretation of data. MA. B. and S. S support the study. M.B. supervised the study and revised the manuscript. All authors read and approved the final manuscript.

Ethics declarations

All experiments were conducted following the Guide Use of Laboratory Instruments and approved by the "Ethics Committee of Tarbiat Modares University" IR.MODARES.REC.1398.072**:**

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest related to this study.

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