Turk Kardiyol Dern Ars 2017;45(2):118-125 doi: 10.5543/tkda.2016.82389

ORIGINAL ARTICLE / KLİNİK ÇALIŞMA

Investigating the role of ceramide metabolism-associated CERS5 (LASS5) gene in atherosclerosis pathogenesis in endothelial cells

Seramid metabolizması ile ilişkili CERS5 (LASS5) geninin endotel hücrelerinde ateroskleroz patogenezindeki rolünün araştırılması

Neslihan Çoban, M.D., Filiz Güçlü Geyik, M.D., Özlem Yıldırım, M.D., Nihan Erginel Ünaltuna, M.D.

Department of Genetics, İstanbul University, Aziz Sancar Experimental Medicine Research ınstitute, İstanbul, Turkey

ABSTRACT

Objective: Ceramide, the backbone of sphingolipids, is the key component affecting atherosclerotic changes through its important second-messenger role. Previous studies have demonstrated protective role of AMP-activated protein kinase (AMPK) genes in regulating atherosclerosis and hypertension. Ceramide synthase 5 (LASS5 or CERS5) gene has function in de novo synthesis of ceramide, and has indirect effect on AMPK gene. Aim of the present study was to identify role of LASS5 gene in atherosclerosis.

Methods: LASS5 gene-specific small interfering RNA (siRNA)-mediated gene silencing was performed in human umbilical vein endothelial cells (HUVEC) and differential expression of LASS5, AMPK-alpha and AMPK-alpha target genes were analyzed. HUVEC cells were then treated with AMPK activator in order to examine relationship of change in gene expression levels to AMPK activity.

Results: Novel physiological function of LASS5 was identified. Downregulation of LASS5 was found to attenuate ceramide production and increase expression of some AMPK target genes in HUVEC.

Conclusion: This is the first study to demonstrate that LASS5 was involved in negative regulation of atherosclerosis-related genes, such as AMPK-alpha. These preliminary findings provide insight into molecular mechanism of atherosclerosis and are important for development of potential therapeutic agents in the treatment of atherosclerosis.

ÖZET

Amaç: Spingoliplerin yapısını oluşturan seramid ikincil haberci olarak ateroskleroz patogenezinde önemli rol oynamaktadır. AMPK geninin, düzenlediği genler üzerinden ateroskleroz ve hipertansiyon gibi hastalıklarda koruyucu rol oynadığı gösterilmiştir. De novo seramid sentezinde rol alan LASS5 (CERS5) geninin, AMPK genini dolaylı olarak etkilediği de belirlenmiştir. Bu çalışmanın amacı, aterosklerozda LASS5 geninin rolünü belirlemektir.

Yöntemler: HUVEC'de (human umbilical vein endothelial cells) LASS5 genine özgü siRNA aracılığı ile gen sessizleştirilmesi yapıldı. Gen sessizleştirilmesinin ardından kardiyovasküler hastalıklarla ilişkili olan LASS5, AMPK-Alfa ve AMPK-Alfa hedef genlerinin hücresel gen ekspresyon seviyelerindeki değişimler araştırıldı. LASS5 geninin baskılanması sonucundaki ekspresyon değişikliğinin AMPK aktivitesindeki değişime bağlı olup olmadığını anlamak için, HUVEC kültür ortamına AMPK aktivatörü eklendi.

Bulgular: LASS5 geninin yeni bir fizyolojik fonksiyonu belirlendi. LASS5 geninin susturulması, HUVEC hücre hattında AMPK metabolizması ile ilişkili genlerin mRNA ekspresyonunu değiştirdi.

Sonuç: Bu çalışma ile ilk defa LASS5 geninin ateroskleroz ile ilişkili olan AMPK-alfa geninin negatif düzenlemesinde yer alabileceği gösterilmiştir. Bu bulgular, aterosklerozun moleküler mekanizmasının anlaşılmasında bir adım niteliği taşımakta olup ayrıca aterosklerozun tedavisinde kullanılabilecek maddelerin geliştirilmesi için ön çalışma olarak önem taşımaktadır.

Sphingomyelin pathway is a phylogenetically preserved signal conduction pathway just like cAMP, and phosphoinoside pathways. In this pathway secondary messenger ceramide are produced from sfingomyelins under the effects of or sfingomyelinase or they are synthesized de novo by ceramide synthase.[1] Important role of ceramide- mediated signal pathway in ischemia, insulin resistance, diabetes, atherogene-

Received: June 30, 2016 *Accepted:* November 24, 2016

Correspondence: Dr. Neslihan Çoban. İstanbul Üniversitesi Deneysel Araştırma Enstitüsü, Genetik Anabilim Dalı, Vakıf Gureba Caddesi, 34280 İstanbul, Turkey.
Tel: +90 212 - 414 20 00 e-mail: neslic@istanbul.edu.tr

sis, septic shock, and ovarian failure has been demonstrated in biochemical, and clinical studies.[2]

It is already known that increased free fatty acids levels due to obesity, and excess food intake, also increase TNF-alpha, and glucocorticoid levels, intracellular lipid metabolites leading to the development of insulin resistance.[3] However important role of ceramide in insulin resistance developing under the influence of inflammatory cytokines, and dietary stimulants as saturated fatty acids has been also demonstrated.[2,4–6] Ceramide stimulates eNOS activity in vascular endothelial cells irrespective of the calcium levels, so ceramide is also an important lipid in the development of hypertension, and atherosclerosis.[7]

Ceramide accumulating in cells is thought to be toxic for many types of cells (ie. pancreas □-cells, and cardiomyocytes), and consequently plays a role in the pathogenesis of diabetes, hypertension, heart failure, and atherosclerosis.[2,8] Many biochemical studies have demonstrated its importance in the process of apoptosis, its relationship with increased LDL cholesterol level, and its possible effects on atherosclerosis, ischemia, hypertension, and coronary heart disease.[9,10] Although metabolism of ceramide has been the subject of many biochemical investigations, it has not been analyzed from genetic perspective. LAG1 (longevity assurance homolog 5 - LAG1) homologue which is known to be responsible from de novo ceramide synthesis is suggested to be a suitable candidate for the comprehension of molecular mechanism underlying cardiovascular diseases.

As a member of Lag (Longevity assurance gene) gene family LASS5 is a 37.5 kb –long gene with 10 exons localized at 12q13.13. It encodes a protein on transmembrane region which involves Hox DNA binding site localized on endoplasmic reticulum, and nuclear membrane. The role of LASS gene in cardiovascular diseases is not known. In a study performed, the indirect association between AMP- sensitive protein kinase gene (AMPK) which is already known to be associated with cardiovascular risk factors has been demonstrated.[11] In this study it haas been revealed that AMPK inhibitor Compound C (Dor-somorhine: 6-[4-(2-Piperidin-1- ylethoxy)phenyl]-3-pyridin-4-ylpyrazolo[1,5-a] pyrimidine, AMPK Inhi-bitör) increases synthesis of ceramide with an impact on LASS5 gene.[11] However recent studies have determined that in mice where expression of LASS5 gene decreases (knock-out mice) in line with decrease with C16 ceramide levels, incidence of obesity, and obesity –related diseases.[12]

In a separate study performed in mice, it has been disclosed that deficiency of sortilin gene leads to decrease in the expression of LASS5 gene responsible from synthesis of ceramide with ameliorative effects on obesity, and insulin resistance.[13]

Kısaltmalar:

AICAR	*AMPK activator*
AMPK	*AMP-activated protein kinase*
eNOS	*Endothelial nitric oxide synthase*
GADPH	*Endojenous control*
GFP	*Green fuorescentprotein*
HUVEC	*Human umbilical vein endothelial cells*
KLF2	*Krüppel-like Factor 2*
LASS/CerS	*Longevity assurance homologue/ ceramide synthase*
MAPK	*Mitogen-activated protein kinase*
qPCR	*Quantitative polymerase chain reaction*
siRNA	*Small interfering RNA*

Since LASS5 gene plays a role in de novo synthesis of ceramide, and its one of clones retrieved from the library of 'subtractive' hybridization cDNA LASS5 gene has been predicted to be an important candidate gene in the heart diseases.[14] Endothelial cells lead the way among cells playing a key role ın the development of atherosclerosis. Therefore realization of this study on endothelial cells was planned. In this study especially in cases where LASS5 gene is suppressed by "small interfering RNA" (siRNA) in the endothelial cell culture, and in the presence of AICAR (5- Aminoimidazole-4-carboxamide 1-□-D-ribofuranoside, Acadesine, N1-(□-D-Ribofuranosyl)-5-aminoimidazole-4-carboxamide) which is determined as an activator of AMPK that is both associated with cardiovascular diseases, and suppressed by ceramide synthesis, we investigated whether expression of AMPK-alfa-1 gene is effected. In addition, in conditions where LASS5 gene is suppressed, the changes in the expressions of target genes of AMPK linked with atherosclerosis were investigated.

With this method we aimed to investigate the role of LASS5 gene in conditions as atherosclerosis, and hypertension by analyzing AMPK in cell cultures.

METHODS

Sequencing of endothelial cells

In this study HUVEC (Human Umbilical Vein Endothelial Cells) (ATCC No. CRL-1730) cell sequences were used. General characteristics of these strains of cells are given below:

HUVEC cell sequences were isolated from normal umbilical veins of human beings. When examined under light microscope, they appear as cells adhered to the surface of the Petri plates. Nearly 24 hours after incubation, endothelial cells increase 2-fold. These cell strains were used in the study because they elicit responses against stimulation of cytokines during expression of cell adhesion molecules, and especially to investigate LASS5 and AMPK genes due to their effects on atherosclerosis.

Endothelial cell culture

Dissolution procedure of endothelial (HUVEC) cells was completed by firstly keeping the cells in 37°C water bath for nearly 5 minutes, then the cells were transferred into a centrifuge tube containing 5 mL culture media, and centrifuged at 125g for 5-7 minutes. The cells retrieved was balanced with RPMI (Lonza, USA) culture media containing 10% fetal bovine serum (FBS, Sigma, USA) and inoculated on 60x20 mm Petri dishes each containing $1.3x10^5$ viable cells per milllilitre. Endothelial cells were cultivated in a total of 5 mL culture media. The cells inoculated in flasks were left for incubation overnight at 37°C, and under 5% CO_2 pressure. Every day the cells were controlled under light microscope as for cellular density, morphology, and presence of potential infection. Culture media was changed every other day. When cellular density reached to 80%, passaging was performed. Vi-Cell cell counter (Vi-CELLTM XR cell counter: Beckman Coulter Life Sciences) was used to determine cell counts, and viability ratio. Passaging rates were 1:2, and 1:3 dependent on the cell density. When 60x20 mm Petri dishes were passaged at a rate of 1 1:3, then cells were inoculated on 100x20 mm Petri dishes. During this procedure following treatment with trypsin, all of the culture media in the Petri dish was placed in a conic centrifuge tube, and endothelial cells in suspension were centrifuged to form pellets. Then these pellets were suspended with fresh culture media, and inoculated on new Petri dishes.

Determination of siRNA concentrations appropriate for LASS5 gene in endothelial cells

In order to silence LASS5 gene endothelial cell culture was transfected with commercially obtained siRNA. For these tests commercially purchased siRNA kits (Ambion, California, USA) were used Target,and control siRNA sequences were as follows: hLASS5, AAACCCTGTGCACTCTG- TATT, and non-targeting control, AATTCTCCGA- ACGTGTCACGT.

In the experiment siRNA transfection was realized using a commercial kit (Lipofectamine 2000, Invitro- gen, California, USA), and a method based on lipid- mediated transfection After determination of the amount of siRNA which silenced greatest number of genes (>80%) without decreasing cell viability, the essential experiment was performed. To this end, for the determination of effective amount of LASS5 siRNA, many trials were performed within 5 nM-70 nM concentration range. More effective silencing was decided to occur at 70 nM, and the main experiment was performed at that level. During experiments, as positive control siRNA targeting GAPDH gene, as negative control, a siRNA control (Ambion, California) which can not silence any gene known as a control gene were used. The experiments were realized using 24-well Petri dishes, and for each siRNA three wells were used for control. Based on the literature information related to AICAR (Sigma, USA), after trials with different concentrations, the appropriae concentration was determined. The experiment was terminated 24-48 hours later, and the cells scraped from the surface of the Petri dishes were preserved at -80°C in liquid nitrogen using shock- freezing method till isolation of RNA was achieved.

Isolation of RNA and cDNA synthesis

RNA was isolated from frozen, and stored endothelial cell samples in the form of pellet. For isolation of RNA from samples containing RNA at a moderate concentration which also ensures high-quality RNA isolation mini-kits were used (Roche, High Pure RNA Isolation Kit, California, USA). Spectrophotometric measurements were made to determine the quality of RNA samples obtained using Nanodrop (Roche 2000, USA) device. Synthesis of cDNA was realized using total RNAs obtained from samples of endothelial cells and cDNA synthesis kit (Roche, Tanscriptor First Strand cDNA Synthesis Kit, California, USA).

Designing primers for selected genes

Primers were designed for both GADH control gene to be used as a control in the study, and also for LASS5, and AMPK-alfa genes whose expression levels will be investigated. During this procedure which was realized manually, RNA hairpin loop, for primers homo-dimer, and for the investigation of heterodimers

between two primers NCBI Nucleotide (http://www.ncbi.nlm.nih.gov/nuccore/) and UNIGENE (http://www.ncbi.nlm.nih.gov/unigene/) information banks, and Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi), Oligo- analyzer 3.1 (IDT Technologies, http://www.idtdna.com) and GeneWalker (Cybergene AB, http://www. cybergene.se/primerdesign/genewalker/genewal- ker11.html) programs were employed.

In designing primers, we took care to analyse mRNA selected from coding sequence (CDS), and if possible including sequences at exon-exon borders. Besides 18-22 bp –long primers, and sequences with GC ratio of 40-60% were chosen with special emphasize at GC content at 3' terminal. In addition, owing to the characteristics of the sequence, primer dimer, and hairpin loop had to be taken into consideration while designing a primer. Besides we were attentive about increased delta G levels. Sequencing primers were as follows: LASS5 'Forward': 5'-tgggaactctgatcatgtgtcta-3', 'Reverse': 5'-agccgctgatacttggcata-3'. AMPK-Alfa1 'Forward': 5'-gaatggaaggctggatgaaa-3', 'Reverse':5'-ttctggtgcagcatagttgg-3'. GAPDH 'Forward': 5'-tcaccatcttccaggagcgaga-3', 'Reverse'': 5'-tgcaggaggcattgctgatga-3'. eNOS 'Forward: 5'-tggtacatgagcactgagatcg-3', 'Reverse': 5' '-ccacgttgatttccactgctg-3'. KLF2 'For-ward' - agacctacaccaagagttcgcatc-3', 'Reverse': 5'- catgtgccgtttcatgtgcagc-3'.

qRT-PCR tests for target genes of LASS5 and AMPK

RT-PCR was performed using cDNA samples, and gene-specific primers. Then the most optimal conditions for qRT-PCR analyses were determined. In order to determine the degree of suppression with siRNA in concentrations studied, and to detect to what extent LASS5, and AMPK target genes were effected, gene expression analyses were performed using SyberGreen (Roche, California, USA) simultaneous quantitative PCR technique, and LASS5, GAPDH, eNOS, KLF2 and AMPK-Alfa1 –specific primers. The variations detected after comparisons were normalized, and calculated based on GAPDH (endogenous control) transcript levels.

RESULTS

Based on the results of siRNA concentration analyses, and as an outcome of expression analyses at 5 nM, 50 nM ve 70 nM concentrations, we observed that GAPDH control gene, and LASS5 gene were suppressed at a rate of 95, and 70%, respectively. Each condition was analyzed 2 times at 24., and 48. hours. (Fig. 1–3). İn consideration of these outcomes, and as a result of cultures performed with an extended concentration range of s.RNA, and subsequent expression analyses, for the final experiment use of 70 nM concentration was decided upon (Fig. 1, and 2).

The reason for selection of 70 nM concentration is that when suppression rates observed at other concentrations used were compared, the maximum suppression was obtained at 70 nM concentration at 48. hour. Besides, as seen in Figure 3, at this concentration cell counts, and cell viability did not very much changed.

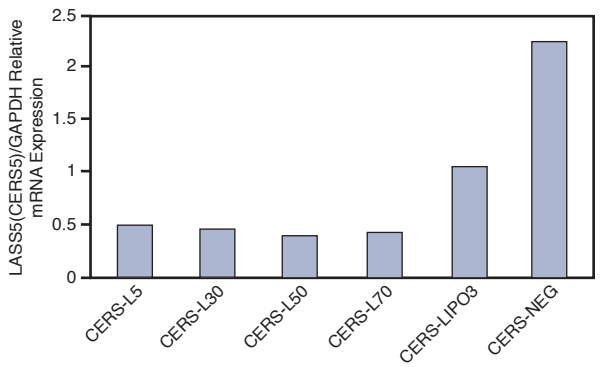

Figure 1. Illustration of suppression rates of siR-NAs of LASS5 (CERS5) and GAPDH control genes between 5 nM-70 nM concentration range as displayed on Real Time PCR and using appropriate expression primersat 24th hour after transfection with 3 µl Lipo-fectamine 2000 (Mock 3 µl).

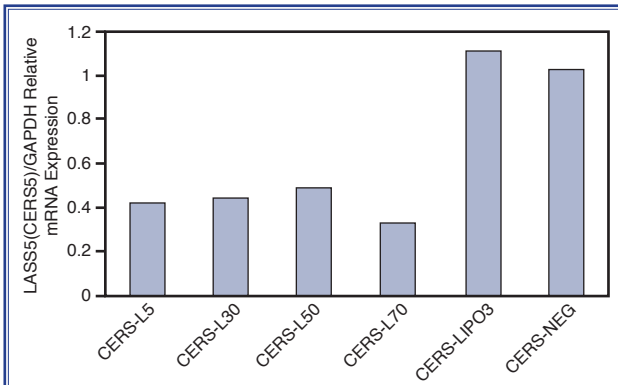

Figure 2. Illustration of suppression rates of siR-NAs of LASS5 (CERS5) and GAPDH control genes between 5 nM-70 nM concentration range as displayed on Real Time PCR and using appropriate expression primers at 48th hour after transfection with 3 µl Lipo-fectamine 2000 (Mock 3 µl).

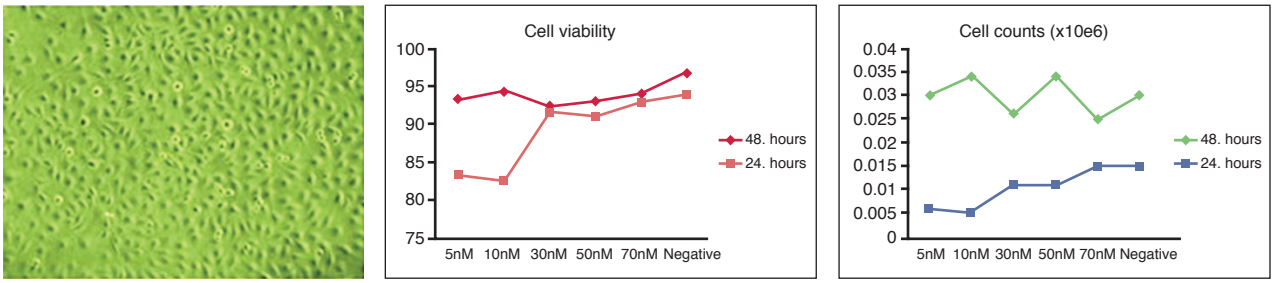

Figure 3. The effect of siRNA of LASS5 (CERS5) gene within 5 nM-70 nM concentration range on cell viability, and cell counts at 24., and 48. hours.

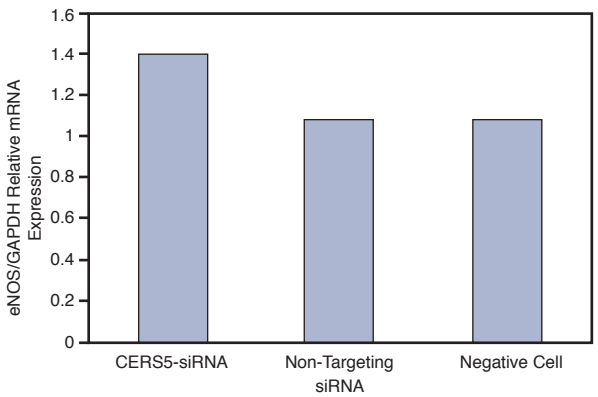

Figure 4. Comparison of expression levels of eNOS gene with those of the negative cell.in conditions where LASS5 (CERS5) gene is suppressed

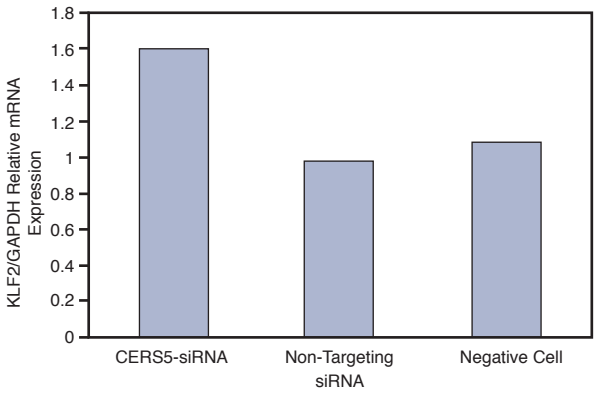

Figure 5. In conditions where LASS5 (CERS5) gene is suppressed, comparison of expression levels of KLF2 gene with those of the negative cell.

The main experiment was started at 70 nM concentration for the analyses of LASS5, and control gene siRNA, and silencing trials were performed at 24., and 48. hours of incubation Based on the outcome of this trial, the degree of impact on AMPK-alfa1 gene expression was determined creating separate conditions for AMPK activator. Decrease in ceramide production with silencing of LASS5 gene led to increase in the expression levels of eNOS, and KLF2 genes (Fig. 4, and 5).

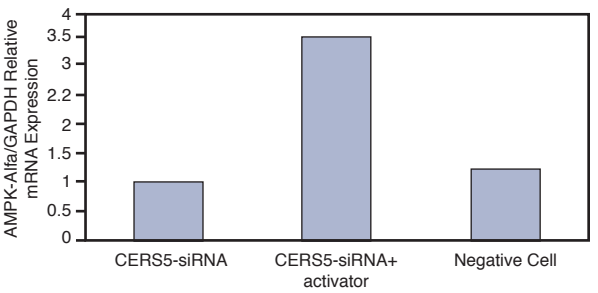

Figure 6. Comparison of expression levels of AMPK-Alfa1 gene In conditions where LASS5 (CERS5) gene is suppressed, and in the presence, and absence of AMPK activator (AICAR).

Stimulation of cells with AMPK activator

At first step, literature studies were reviewed in detail to determine appropriate concentrations of AICAR, and the most optimal concentration (1mM) for AICAR at HUVEC cell line was determined. For further studies to be performed with cell culture 1 mM concentration of this activator was used.

As a result of silencing LASS5 gene, and in the presence of AMPK-Alfa activator, variations in AMPK-Alfa1 gene were analyzed, and findings obtained confirmed our results (Fig. 6). In conditions where LASS5 gene was silenced, extreme variations were not observed in the expression of AMPK-Alfa1 gene when compared with that of negative cells. On the other hand, when AMPK activator (AICAR) was incorporated into silencing process of LASS5 gene, more than 3-fold increase in AMPK-Alfa expression was observed.

DISCUSSION

Atherosclerosis is the basic underlying factor of cardiovascular diseases.[15–17] Atherosclerosis has been defined as an inflammatory disease resulting in the formation of atherosclerotic plaque caused by accumulation of cholesterol on the intimal layer of arteries.[19–21] Genetic analysis of ceramide metabolism whose significance in the pathogenesis of atherosclerosis has been shown in clinical, and biochemical studies conveys utmost importance.

As is known, intracellularly accumulated ceramide is toxic for many cell types (pankres beta cells, and cardiomyocytes), and consequently it plays an important role in the pathogenesis of diabetes, hypertension, heart failure, and atherosclerosis.[22,23] Besides atherosclerotic plaque contains increased amounts of ceramide.[24] Sphingolipids, and their metabolic products (ceramide) are important components which integrate with the structure of cell membrane, and assume a task in the regulation of cellular functions. Ceramide which plays a role in various metabolic pathways is produced from sphingomyelins under the influence of sphingomyelinase or synthesized de novo by ceramide synthase enzyme.[25] LASS5 gene which is known to be responsible from de novo synthesis of ceramide seems to be an appropriate candidate gene for the understanding of underlying molecular mechanisms of cardiovascular diseases. The role of LASS5 gene in cardiovascular diseases has not been known yet. In a recent study, an indirect relationship between LASS4 gene, and AMPK whose association with cardiovascular risk factors is already known has been demonstrated.[11] AMPK is one of the most important types of protein involving in intracellular conduction pathways. It is activated when intracellular ATP levels decrease, and AMP levels increase, and generally it suppresses anabolic pathways with resultant increase in the production of ATP.[26] In a study performed on AMPK inhibitor the mechanism of suppression of AMPK by compound C was investigated.[11] In this study compound C has been demonstrated to suppress AMPK by increasing synthesis of ceramide, Also LASS5 gene was found to be responsible from increased synthesis of ceramide.[11] Our results also support the findings of their study. In conditions where LASS5 gene is silenced increases in the expressions of eNOs, and KLF2 which are target genes of AMPK were observed when compared with the expression of negative cells. In the presence of siRNA when an AMPK activator was added to the process, more than 3- fold increase was observed in the expression of AMPK-Alfa1 In studies on AMPK protein, stimulation of eNOS activity by resveratrol has been associated with, and also dependent on AMPK[27,28] In another study, stimulation of KLF2 expression in endothelial cells by blood flow has been shown to be mediated by AMPK.[29,30] Based on the results of all these studies, it has been thought that LASS5 levels, and activity may play a significant role on the effects of AMPK related to the risk of cardiovascular risk According to our study results. İt has been suggested that LASS5 gene which is responsible from synthesis of ceramide exerts its indirect effects on eNOS, and KLF2 genes which have very important effects in atherosclerosis, and hypertension by way of AMPK.

These outcomes suggest that increase in the expression of LASS5 gene which is responsible from synthesis of ceramide in endothelial cells which are important cell types at the onset, and advanced stages of atherosclerosis may be achieved with changes in AMPK-Alfa pathway. When LASS5 gene is silenced, expression levels of genes targeted by AMPK may change suggesting important role of LASS5 gene in the pathogenesis of atherosclerosis.

As for cardiovascular diseases, the correlation between AMPK, and ceramide levels has been analyzed in many studies,[32,33] however genetic basis of this disease has not been studied in many studies. In a recent study it has been suggested that ceramide levels are modified through phosphorilation of AMPK over micro RNAs, the underlying mechanism of decrease in obesity, and type 2 diabetes mellitus via phosphorylation of myocardial AMPK has not been fully understood yet.[34]

These preliminary results of ours demonstrate that LASS5 gene may be one of the control mechanisms of endothelium which is atherosclerotic cell type. However to arrive at more definitive results, larger-scale comprehensive studies on metabolic pathways of investigated target genes should be performed. In order to prove the results we obtained, priorly, in the presence of AMPK ligands, the time span where ligands remain active, and the actual time span where AMPK gene expression is effected should be deter-

mined. Besides it is possible to perform more detailed investigations by increasing number of target genes. Afterwards, protein analyses should be conducted to determine the effect of gene expression on the levels of proteins. Besides the results should be confirmed by more detailed analyses as measurement of ceramide synthase activity, and mass spectrometry studies. Further confirmation of the results in animal studies will facilitate their clinical applicabilities. In vivo confirmation of the relation between cellular changes in atherosclerotic plaques of human beings, and LASS5, and AMPK activities may convey major clinical implications. Studies performed with the biomaterials harvested directly from patients specimens may provide clear-cut information on this issue.

Acknowledgement

This study was supported by, TÜBITAK health Sciences Research Support Group. Project No: 115S139. In addition, this study was supported by Scientific Research Projects Coordination Unit of Istanbul University (contract grant numbers: 33021 and 52989).

Conflict-of-interest issues regarding the authorship or article: None declared

REFERENCES

1. Mathias S, Peña LA, Kolesnick RN. Signal transduction of stress via ceramide. Biochem J 1998;335:465–80.
2. Summers SA, Nelson DH. A role for sphingolipids in producing the common features of type 2 diabetes, metabolic syndrome X, and Cushing's syndrome. Diabetes 2005;54:591–602.
3. Grigsby RJ, Dobrowsky RT. Inhibition of ceramide production reverses TNF-induced insulin resistance. Biochem Biophys Res Commun 2001;287:1121–4.
4. Summers SA. Ceramides in insulin resistance and lipotoxicity. Prog Lipid Res 2006;45:42–72.
5. Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, et al. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. Cell Metab 2007;5:167–79.
6. Zierath JR. The path to insulin resistance: paved with ceramides? Cell Metab 2007;5:161–3.
7. Igarashi J, Thatte HS, Prabhakar P, Golan DE, Michel T. Calcium-independent activation of endothelial nitric oxide synthase by ceramide. Proc Natl Acad Sci U S A 1999;96:12583–8.
8. Pickersgill L, Litherland GJ, Greenberg AS, Walker M, Yeaman SJ. Key role for ceramides in mediating insulin resistance in human muscle cells. J Biol Chem 2007;282:12583–9.
9. Argaud L, Prigent AF, Chalabreysse L, Loufouat J, Lagarde M, Ovize M. Ceramide in the antiapoptotic effect of ischemic preconditioning. Am J Physiol Heart Circ Physiol 2004;286:246–51.
10. Ichi I, Nakahara K, Miyashita Y, Hidaka A, Kutsukake S, Inoue K, et al. Association of ceramides in human plasma with risk factors of atherosclerosis. Lipids 2006;41:859–63.
11. Jin J, Mullen TD, Hou Q, Bielawski J, Bielawska A, Zhang X, et al. AMPK inhibitor Compound C stimulates ceramide production and promotes Bax redistribution and apoptosis in MCF7 breast carcinoma cells. J Lipid Res 2009;50:2389–97.
12. Gosejacob D, Jäger PS, Vom Dorp K, Frejno M, Carstensen AC, Köhnke M, et al. Ceramide Synthase 5 Is Essential to Maintain C16:0-Ceramide Pools and Contributes to the Development of Diet-induced Obesity. J Biol Chem 2016;291:6989–7003.
13. Rabinowich L, Fishman S, Hubel E, Thurm T, Park WJ, Pewzner-Jung Y, et al. Sortilin deficiency improves the metabolic phenotype and reduces hepatic steatosis of mice subjected to diet-induced obesity. J Hepatol 2015;62:175–81.
14. Komurcu E. Subtractive hibridizasyon yöntemi ile kalbe özgü cDNA kütüphanesinin oluşturulması. İstanbul Üniversitesi Deneysel Tıp Araştırma Enstitüsü, Genetik Anabilim Dalı, İstanbul, Yüksek Lisans Tezi, 2002.
15. Castelli WP. Lipids, risk factors and ischaemic heart disease. Atherosclerosis 1996;124 Suppl.: 1–9.
16. Falk E, Fuster V. Atherogenesis and its Determinants. In: Fuster V, Alexander RW, O'Rourke RA, editors. Hurst's The Heart. 10th ed. USA. International Edition: McGraw-Hill Medical Publishing Division; 2001. p. 1065–93.
17. Fruchart JC, Nierman MC, Stroes E, Kastelein J, Duriez P. New risk factors for atherosclerosis and patient risk assessment. Circulation 2004;109:15–9.
18. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135–43.
19. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 2006;6:508–19.
20. Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque: part I: evolving concepts. J Am Coll Cardiol 2005;46:937–54.
21. Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the vulnerable plaque. J Am Coll Cardiol 2006;47(8 Suppl):13–8.
22. Aerts JM, Ottenhoff R, Powlson AS, Grefhorst A, van Eijk M, Dubbelhuis PF, et al. Pharmacological inhibition of glucosylceramide synthase enhances insulin sensitivity. Diabetes 2007;56:1341–9.
23. Adams JM 2nd, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, et al. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. Diabetes 2004;53:25–31.
24. Edsfeldt A, Dunér P, Ståhlman M, Mollet IG, Asciutto G, Grufman H, et al. Sphingolipids Contribute to Human

Atherosclerotic Plaque Inflammation. Arterioscler Thromb Vasc Biol 2016;36:1132–40.
25. Wegner MS, Schiffmann S, Parnham MJ, Geisslinger G, Grösch S. The enigma of ceramide synthase regulation in mammalian cells. Prog Lipid Res 2016;63:93–119.
26. López M, Nogueiras R, Tena-Sempere M, Diéguez C. Hypothalamic AMPK: a canonical regulator of whole-body energy balance. Nat Rev Endocrinol 2016;12:421–32.
27. Xu Q, Hao X, Yang Q, Si L. Resveratrol prevents hyperglycemia-induced endothelial dysfunction via activation of adenosine monophosphate-activated protein kinase. Biochem Biophys Res Commun 2009;388:389–94.
28. Bonnefont-Rousselot D. Resveratrol and Cardiovascular Diseases. Nutrients 2016.
29. Cui X, Liu X, Feng H, Zhao S, Gao H. Grape seed proanthocyanidin extracts enhance endothelial nitric oxide synthase expression through 5'-AMP activated protein kinase/Surtuin 1-Krüpple like factor 2 pathway and modulate blood pressure in ouabain induced hypertensive rats. Biol Pharm Bull 2012;35:2192–7.
30. Young A, Wu W, Sun W, Benjamin Larman H, Wang N, Li YS, et al. Flow activation of AMP-activated protein kinase in vascular endothelium leads to Krüppel-like factor 2 expression. Arterioscler Thromb Vasc Biol 2009;29:1902–8.
31. Weikel KA, Cacicedo JM, Ruderman NB, Ido Y. Glucose and palmitate uncouple AMPK from autophagy in human aortic endothelial cells. Am J Physiol Cell Physiol 2015;308:249–63.
32. Ishibashi Y, Kohyama-Koganeya A, Hirabayashi Y. New insights on glucosylated lipids: metabolism and functions. Biochim Biophys Acta 2013;1831:1475–85.
33. Erickson KA, Smith ME, Anthonymuthu TS, Evanson MJ, Brassfield ES, Hodson AE, et al. AICAR inhibits ceramide biosynthesis in skeletal muscle. Diabetol Metab Syndr 2012;4:45.
34. Lopaschuk GD, Ussher JR. Targeting microRNAs to limit myocardial lipid accumulation. Circ Res 2015;116:229–31.

Keywords: AMPK; atherosclerosis; ceramide; LASS5; siRNA.

Anahtar sözcükler: AMPK; ateroskleroz; seramid; LASS5; siRNA.