



Evaluation of Sestrin 2, Adiponectin, AMPK, and mTOR Genes Expression in Acute Myeloid Leukemia Patients

Seyed Hossein Abtahi¹, Mohammad Hossein Mohammadi^{1,2}, Mehdi Allahbakhshian Farsani^{1,2*}, Zahra Aghelan³, Sina Salari⁴

¹ Department of Laboratory Hematology and Blood Bank, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

² HSCT Research Center, Shahid Beheshti University of Medical Science, Tehran, Iran.

³ Department of Clinical Biochemistry, School of Medical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran

⁴ Department of Medical Oncology, Hematology and Bone Marrow Transplantation, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

*Corresponding author: Mehdi Allahbakhshian Farsani, Department of Laboratory Hematology and Blood Bank, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. HSCT Research Center, Shahid Beheshti University of Medical Science, Tehran, Iran. School of Allied Medical Sciences, Quds Sq, Tajrish, Tehran, Iran. Tel/Fax: +98-2122717504, E-mail: allahbakhshian@sbmu.ac.ir

Background: Effective treatment of acute myeloid leukemia (AML) is still controversial, therefore; a comprehensive understanding regarding the impaired cellular signaling pathways in AML can be useful in designing new therapeutic approaches. Among signaling pathways involved in AML, the mammalian target of rapamycin (mTOR) signaling pathway is of particular importance. While dysregulation of mTOR signaling has been reported in a wide range of patients with AML, but most studies have focused on mTOR downstream targets, and mTOR upstream targets have been overlooked.

Objective: In this study, expression of mTOR genes and three upstream targets (5' adenosine monophosphate-activated protein kinase (AMPK), adiponectin, and sestrin 2) involved in mTOR signaling was investigated.

Materials and Methods: In this study, expression of mTOR, AMPK, sestrin 2, and adiponectin genes in 60 patients with AML were evaluated compared to those of 30 healthy individuals as controls using the Real-Time polymerase chain reaction (Real-Time RT-PCR) method.

Results: According to the results, there was a significant difference in the expression of all the studied genes in patients in comparison to the normal control group ($P < 0.05$). Expression of the mTOR gene was increased, while expression of AMPK, sestrin 2, and adiponectin genes was decreased in the patients with AML. Mean expression of the genes ($2^{-\Delta Ct}$) (AMPK, sestrin 2, adiponectin, and mTOR) was equal to 7.9, 3.2, 3.74, and 1.49 for controls and 6, 2.1, 2.83, and 2.64 for patients with AML, respectively.

Conclusions: Given the decreased expression levels of sestrin 2, adiponectin, and AMPK genes as tumor inhibitors and the increased expression level of the mTOR gene as an oncogene in the patients with AML in our study, it is thought that disruption of this pathway may be involved in leukemogenesis and can be considered as an effective factor in the progression of cancer.

Keywords: Adiponectin, AML, AMPK, mTOR, and Sestrin 2

1. Background

Acute myeloid leukemia (AML) is characterized by invasive proliferation and abnormal differentiation of hematopoietic stem cells (HSCs) leading to the accumulation of immature myeloid cells with reduced differentiation potential (1). Evidence suggests that various deviations in the cell signaling pathway lead to cessation of maturation of myeloid progenitors (2-4). Mammalian target of rapamycin (mTOR) signaling is

a vital pathway in the regulation of cellular processes including the metabolism of proteins, carbohydrates, and lipids (5, 6). In leukemic cells, mTOR signaling activation is a mandatory prerequisite to reduce growing needs for energy and raw materials. Recent findings have suggested that AML cells are dependent on the increased activity of the glycolysis pathway due to high mTOR activity, and this phenotype may be involved in their sensitivity to inhibition of glucose-6-phosphate

dehydrogenase (G6PD) (7). These data, together with other related studies indicate an antileukemic potential for specific mTOR inhibitors, such as rapamycin analogs (rapalogs) in the patients with AML and provide a significant approach for further studies in this field (8, 9). However, mTOR inhibitors have not been able to show significant results in clinical trials on AML (10, 11).

Determining which patients benefit from which therapies is the ultimate aim of the targeted therapy however, AML therapy has largely failed to achieve this aim, possibly due to bypassing of the damaged signaling pathways by the uncontrolled molecules. For accurately evaluating mTOR as a drug target and using it in a clinical context, it is essential to know all the elements involved in mTOR signaling. In this regard, some studies have recently identified adiponectin and sestrin 2 as the most important negative regulators of the mTOR pathway, which may be due to the activity of AMP-activated protein kinase (AMPK) (12-15). AMPK is a pivotal sensor of cellular energy and acts as the main negative regulator of metabolic pathways including proteins, carbohydrates, and lipids to minimize consumption of adenosine triphosphate (ATP). AMPK controls the rate of protein synthesis through suppression of mTOR signaling, an axis that is impaired in patients with AML (16, 17).

Unlike sestrin 2, which remains in the cytoplasm of producer cells after translation, adiponectin is secreted to plasma and is generally in a circulating form. Adipocytes of bone marrow adipose tissue (MAT) are one of the major secretors of adiponectin. Adiponectin has versatile functions and is involved in the metabolism of lipids, insulin sensitization, and anti-inflammatory responses. In particular, recent studies have shown growth inhibitory effects of adiponectin on hematopoietic cells, produced by activation of AMPK (12-15). So, it appears that adiponectin acts as a tumor inhibitor through the AMPK axis (18, 19). As mentioned above, sestrin 2, a stress-responsive protein responsible for adapting cells to various metabolic challenges is another arm for activation of AMPK (20).

2. Objectives

In this study, for detecting dysregulation of the mTOR pathway in the patients with AML, the expression of the three major regulators of this gene (sestrin 2, adiponectin, and AMPK) was evaluated.

3. Materials and Methods

3.1. Patients

From 2013 to 2017, peripheral blood (PB) and bone

marrow (BM) samples collected from 60 patients with new AML aged 15-72 years (mean 53 years) and 30 PB and BM samples from healthy controls. Informed consent in agreement with the Declaration of Helsinki was confirmed in all patients or parent/legally authorized representatives. Patients were diagnosed at the Taleghani hospital, Tehran, Iran. The acute myeloid leukemia diagnosis was based on the morphological classification of FAB /WHO, which was categorized in M0/M1/M2=29, M3=9, and M4/M5=22 (Demographic and subclinical characteristics of patients samples are summarized in **Table 1**).

3.2. RNA Extraction and cDNA Synthesis

Using RNeasy kit (Total RNA Purification from Whole Blood, Qiagen, Germany) extraction and purification of RNA from samples of peripheral blood and bone marrow mononuclear cells were performed according to the manufacturer's protocol. By Nanodrop (ratio of 260/280 nm OD > 1.8) quality and quantity extracted RNA was assessed. In the next step, cDNA synthesis was performed using a Thermo Scientific kit (Qiagen, USA).

3.3. Real-Time RT-PCR

Primers of target genes (mTOR, AMPK, sestrin 2, and adiponectin) and internal control genes (ABL) were designed using software Oligo. (details are shown in **Table 2**), by real-time quantitative polymerase chain reaction (Real-Time RT-PCR) (Step One Plus, Thermo Scientific, USA) the level of mRNA expression of selected genes was analyzed (**Table 3**). The total volume

Table 1. Profile of specifications of patients with de novo AML from which samples were obtained.

Sex	
Male	38(%63)
Female	22(%37)
Age (years)	
Median	53
Range	15-72
<40	8 (%14)
40-55	18 (%30)
>55	34(%56)
Blasts count	
Median	(% 63.62)
Range	(% 20-96)
Subtypes of FAB/WHO	
M0/M1/M2	29(%48)
M3	9(%14)
M4/M5	22(%38)
Specimen type	
B.M	37(%62)
P.B	23(%38)

Table 2. Real-time RT-PCR oligonucleotide primers.

AMPK	Sequence (5'→3')	TM(°C)	product length
F primer	ATGGTGATGGAATATGTCTCAG	55.3	113
R-primer	TCCACACCAGAAAGGATCT	55.18	
Sesn 2			
F primer	CTGCGTCTTTGGCATCAGATA	58.45	125
R-primer	ACATTCTTCGGGTGGTCTTCT	59.02	
Adiponectin			
F primer	GAGATCCAGGTCTTATTGGTC	55.19	139
R-primer	AATGCTGAGCGGTATACATAG	55.37	
mTOR			
F primer	GCATGAATCGGGATGATCG	56.35	119
R-primer	CTGCTGCTGTGTGATTTCTT	56.63	
ABL			
F primer	TGGAGATAACACTCTAAGCATAACTAAAG	59.13	124
R-primer	GATGTAGTTGCTTGGGACCCA	60.00	

Table 3. Schedule time and temperature of Real-time RT-PCR target genes and reference gene

Stage	Step	Temperature C	Time	Cycle
Holding	Denaturation and enzyme activation	95	10 min	1
	Denaturation	95	10 sec	
Cycling	Annealing	58-64	20 sec	40
	Extraction	72	30 sec	
Holding	Final Extraction	72	10 min	1
Melting	Melting curve analysis	70-99	7 min	1

of 15 µL of components in Real-time RT-PCR reaction for each target was composed of 1 µL of forward and reverse primer, 7.5 µL of Biofact 2X Real-Time PCR Master Mix Sybergreen (Biofact, South Korea), 2 µL of template target cDNA, and 4.5 µL water. A standard curve for each target gene using four dilutions of cDNA sample (1, 0.1, 0.01, and 0.001) was produced. Assessments were performed in triplicate and the Livak method ($2^{-\Delta\Delta Ct}$) for each sample was calculated using the relative amount of mRNA expression (fold change=FQ) (21-22).

3.4. Statistical Analysis

For statistical analysis, SPSS software for Windows (version 24.0) and GraphPad Prism 8.4 software was used. To assess the normal distribution of data in AML patients and controls from both the Shapiro-Wilk and Kolmogorov-Smirnov tests were used. Also, the Student t or Man-Whitney U test was used to determine that there was a significant difference in the expression of target genes between AML and control patients. Finally, the Pearson correlation test was used to investigate the relationship between the expression of target genes. To determine significant differences in the genes assess, P-Value less than 0.05 was considered.

4. Results

4.1. mTOR, AMPK, Sestrin 2, and Adiponectin Expression in AML Patients and Normal Controls

Expression levels of mTOR, AMPK, sestrin 2, and adiponectin were analyzed using the Real-Time RT-PCR method. Normal expression levels of mTOR, AMPK, sestrin 2, and adiponectin were equal to 0.99 – 1.9, 7 - 8.8, 2.7- 3.7, and 3.1-4.2, respectively, which were defined as 95% confidence interval range in normal controls. According to this reference level, the patients with AML whose mTOR, AMPK, sestrin 2, and adiponectin expression was within the normal range were considered to have an intermediate expression (10, 30, 12, and 12%, respectively). While those with levels below the threshold of intermediate-range for mTOR, AMPK, sestrin 2, and adiponectin expression were defined to have low expression levels observed in 29, 65, 64, and 70% of AML patients, respectively. contrarily, high-level expressions were observed in 61, 5, 24, and 18 % of AML-positive patients, respectively. The mRNA expression levels of mTOR, AMPK, sestrin 2 and adiponectin in AML patients were compared with control group. A significant mTOR expression difference between AML patients 2.73 ± 0.2 (SEM)

compared with control group 1.49 ± 0.16 (SEM) ($P < 0.02$). Statistically significant AMPK expression difference was observed between AML patients 6 ± 0.3 (SEM) compared with control group 8 ± 0.26 (SEM) was confirmed by significant level ($P < 0.0003$). Observed difference between sestrin 2 mRNA in AML patients 2.2 ± 0.4 (SEM) compared to control samples 3.24 ± 0.26 (SEM) was confirmed by significant level ($P < 0.01$). A significant adiponectin mRNA difference between patient and control groups was detected, 2.8 ± 0.2 (SEM) and 3.7 ± 0.27 (SEM) respectively ($p < 0.002$) (**Fig. 1** and **Table 3**).

4.2. Correlation between mTOR, AMPK, Sestrin 2, and Adiponectin Expression Levels

Results of statistical analysis show a positive and significant correlation between expression levels of the mTOR gene with those of AMPK and sestrin 2 genes in the patients with AML. Analysis results determined a positive and significant correlation between mTOR and AMPK and sestrin 2 ($P < 0.004$, < 0.001 and $r = 0.37$, 0.42 , respectively) in the patients with AML,

suggesting dependence between their expression. Also, there was no significant correlation between mTOR and adiponectin in the patients with AML (**Fig. 2**).

4.3. Differential Expression of mTOR, AMPK, Sestrin 2, and Adiponectin in AML FAB Subtypes (M0/M1/M2, M3, and M4/M5)

According to the obtained findings, there was no significant difference in the expression level of mTOR, AMPK, and sestrin 2 genes between AML FAB subtypes (M0/M1/M2, M3, and M4/M5).

The mRNA expression levels of mTOR, AMPK, sestrin 2, and adiponectin in AML FAB subtypes (M0/M1/M2, M3, and M4/M5) were compared with the control group. Observed mTOR mRNA difference between M0/M1/M2 2.64 ± 0.2 (SEM), M3 2.83 ± 0.4 (SEM) and M4/M5 2.57 ± 0.29 (SEM) wasn't significant between these subgroups. Observed AMPK mRNA difference between M0/M1/M2 6.3 ± 0.36 (SEM), M3 5.8 ± 0.52 (SEM) and M4/M5 5.7 ± 0.33 (SEM) wasn't significant between these subgroups. Observed sestrin 2 mRNA difference between M0/M1/M2 2.22 ± 0.27 (SEM), M3 2.3 ± 0.4 (SEM) and M4/M5 2.03 ± 0.29 (SEM) wasn't significant

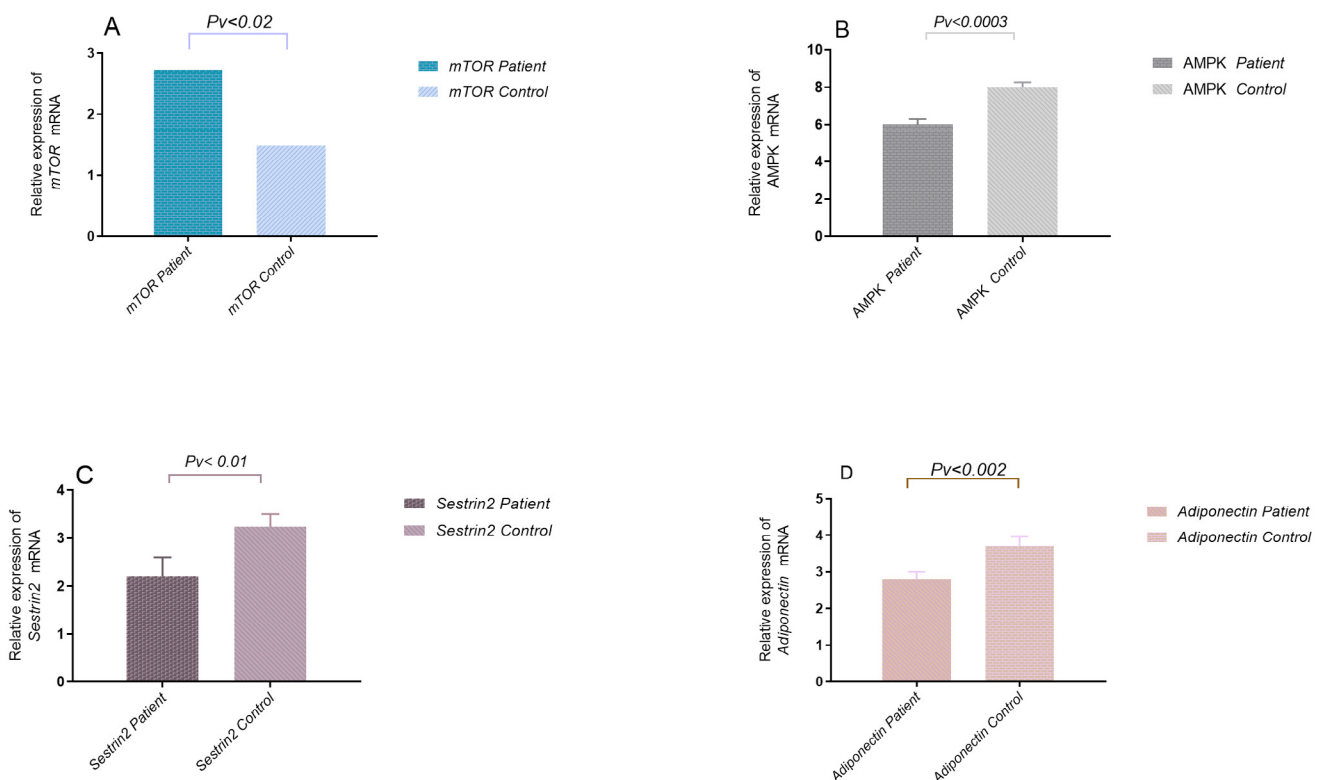


Figure 1. A) Significant mTOR expression difference between patient and control groups. B) Significant AMPK expression difference between patient and control groups. C) Significant sestrin 2 expression difference between patient and control groups. D) Significant adiponectin expression difference between patient and control groups.

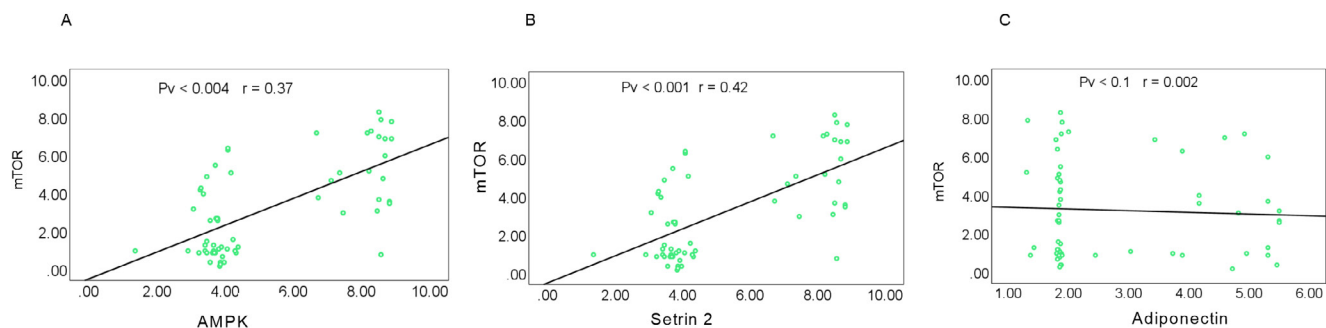


Figure 2. A) Correlation between mTOR and AMPK was determined to be positive and significant in patients. B) Correlation between mTOR and sestrin 2 was determined to be positive and significant in patients. C) Correlation between mTOR and adiponectin was not significant in patients.

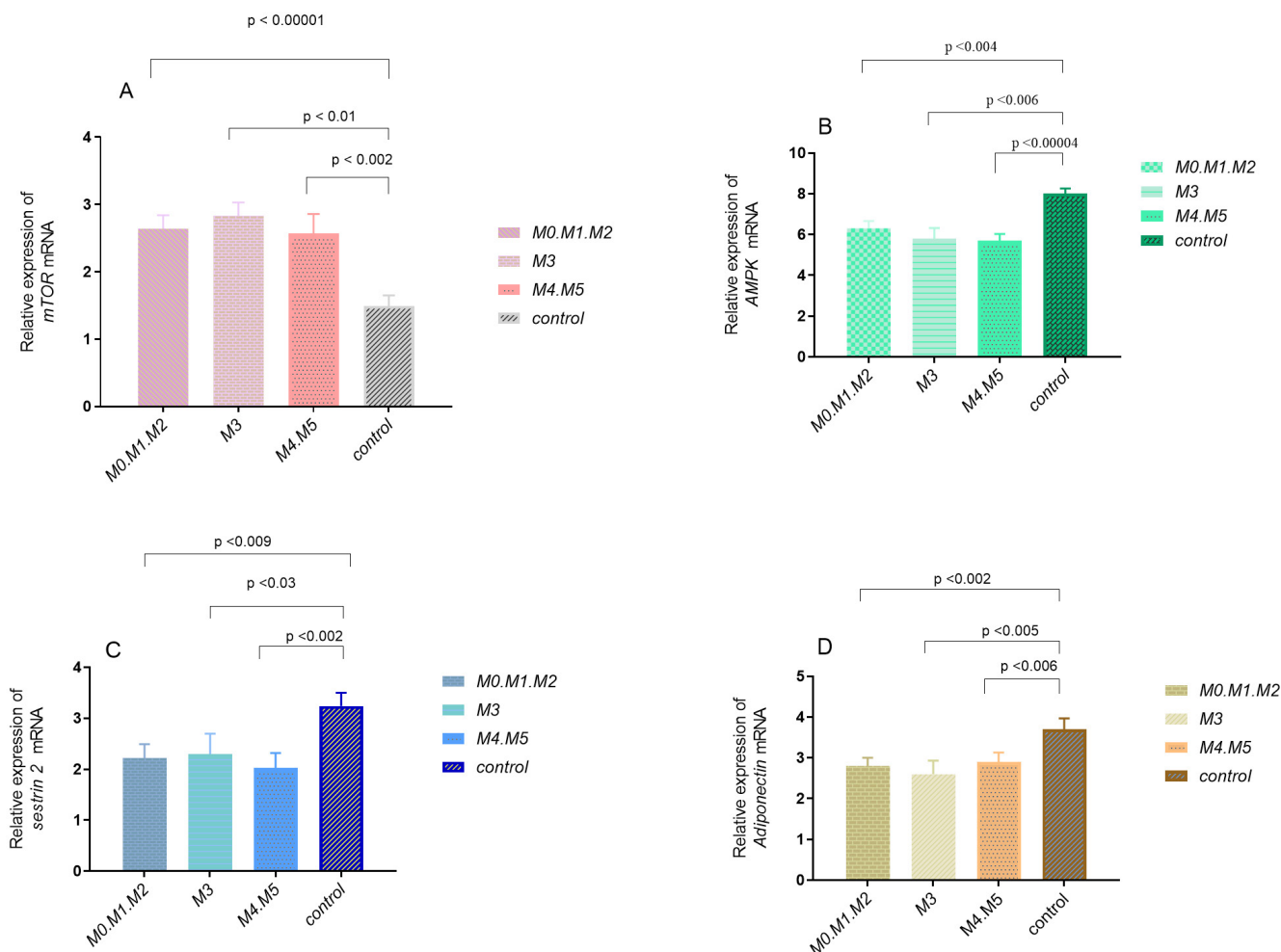


Figure 3. A) mTOR expression difference between AML FAB subtypes wasn't significant between these subgroups. B) AMPK expression difference between AML FAB subtypes wasn't significant between these subgroups. C) Sestrin 2 expression difference between AML FAB subtypes wasn't significant between these subgroups. D) Adiponectin expression difference between AML FAB subtypes wasn't significant between these subgroups.

between these subgroups. Adiponectin mRNA difference between M0/M1/M2 2.8 ± 0.2 (SEM), M3 2.6 ± 0.33 (SEM) and M4/M5 2.9 ± 0.23 (SEM) wasn't significant between these subgroups (**Fig. 3**).

5. Discussion

Under steady-state, coordinated sets of intracellular signaling pathways act to shape cellular architecture. However, all living organisms occasionally face

diverse environmental stresses, such as temperature shock, oxygen alternation, nutrient deprivation, DNA damage, and production of reactive oxygen species (ROS) (23,24). In this situation, they should decide to adopt one of the possible fates including a program to repair and adapt or apoptosis (25). The decision of cells is influenced by both severity of the inflicted stresses and also the pre-event status of cells. During evolution, malignant cells choose survival options while encountering stressful conditions by deactivating apoptosis-inducing pathways. Most recently, in line with this notion, Ghiraldeli *et al.*, (26) demonstrated that primary AML cells with partial AMPK activity were resistant to damage inflicted by chemotherapy in comparison with AML cells with normal AMPK activity. This feature was attributed to the loss of the ability of these cells to produce H2A histone family member X (H2AX), p53, and p21 following exposure to chemotherapy-inducing stresses. These findings have been obtained through laboratory studies and their clinical value depends on the prevalence of AML in the patients showing low or normal AMPK activity. Given the importance of dysregulation of the mTOR pathway in the pathogenesis of AML, in this study, expression of mTOR, AMPK, sestrin 2, and adiponectin genes was evaluated in the patients with AML.

Indeed, from a practical point of view, since Real-time RT-PCR is an accessible and feasible technique in most routine clinical practices and from a biological point of view, because in the cells under steady-state condition, (for this study, primary AML cells before administration of treatment and control cells from healthy donors) levels of transcripts largely explain the amount of respective proteins (27), herein, it was attempted to dissect probable changes in the AMPK axis at the transcriptional level.

There were lower levels of AMPK expression in 65% of the patients with AML (concerning 95% confidence interval of the healthy control group) (0.75-fold change, $p < 0.0003$). However, other patients expressed either similar expression (30 %) or higher expression (5%) in comparison with the healthy control group. These results led us to hypothesize that partial activity of AMPK reported in a previous study on AML cells (17) was at least in part due to downregulation of its expression. Our search was also expanded to identify other possible mechanisms of AMPK partial activity in patients with AML. When gene expression of two positive regulators of AMPK (adiponectin and sestrin 2) was analyzed, it was found that both of them were reduced significantly in 70 and 64% of the patients with AML, respectively (0.76-fold change for adiponectin,

$p < 0.002$ and 0.66-fold change for sestrin 2, $p < 0.01$). However, other studies have shown that liver kinase B1 (LKB1) failed to induce AMPK activity in AML cells (17), and to the extent of our knowledge, aberrance in the expression of adiponectin and sestrin 2 genes in the AML patient-derived cells has not been investigated so far. In agreement with our results, the previous reports have demonstrated that expression of sestrin 2 is decreased in different types of solid tumors such as non-small cell lung cancer, colorectal cancer, and hepatocellular carcinoma. Besides, low expression of sestrin 2 has been reported to be remarkably correlated with the advanced tumor stage, metastasis, and poor prognosis (28-30). Moreover, a negative regulatory role of adiponectin has been found for HSPCs and proliferation of progenitor cells and more investigations have highlighted that plasma circulatory level of adiponectin is decreased in diverse types of cancer including breast cancer, hepatocellular, and colorectal cancers (31-33). As already reviewed (34), in response to a variety of adverse conditions, p53 and AMPK strengthened activation of each other in a positive feedback loop mediated by sestrin 2, which led to reversible cell cycle arrest, senescence, and finally, cell death. Furthermore, adiponectin triggers activation of signaling pathway launching AMPK pathway and in turn, strengthening this loop.

By and large, it was found that 36, 18, and 16% of the patients with AML had decreased expression levels in three, two, and one of AMPK axis elements (adiponectin, sestrin 2, and AMPK), respectively, which in sum delineates that at least one of AMPK pathway genes was disrupted in up to 90% of the patients with AML.

In this regard, p53, a prominent AMPK activator has been previously demonstrated to significantly underexpressed in the patients with AML (35), moreover, other mechanisms, such as TP53 mutations and aberrant expression of p53 regulators, have been found to frequently lead to loss of p53 function in AML (34). Based on this evidence, it is suggested that dysfunction of AMPK pathway is highly prevalent in patients with AML and can be regarded as a broad druggable target. Taken together, based on our experiment and parallel results, it was found that various cellular elements inducing AMPK activity including sestrin 2, adiponectin, p53 (36), and LKB1 (17) are abrogated in AML cells, so the use of AMPK direct activator is suggested instead of indirectly targeting this molecule (such as metformin that induces the activity of LKB1) in the patients with AML.

Our results also demonstrated that 61 % of the studied

patients have a significantly higher expression level of mTOR transcript (fold change= 1.8, $p < 0.02$). In contrast to a majority of studies that tried to provide cytotoxicity for AML cells by inhibiting mTOR, results of a recent study have revealed that induction of AMPK activity in AML cells exhibiting mTOR over-activation led to a specific and higher lethal effect than mTOR inhibition. If mTOR overexpression is considered equivalent to mTOR overactivation, then it is postulated that up to 50% of the patients with AML might benefit from AMPK activator-based therapies. However, the previous studies have reported a higher rate of mTOR overactivation in the patients with AML through tracing multiple phosphorylation sites of mTOR targets by western blot technique (37). In this regard, further studies are needed to determine whether the patients with AML having mTOR overexpression at transcript level benefit differently from AMPK activators.

As studied elsewhere, mTOR and AMPK work in opposing ways to maintain metabolic homeostasis and cell growth (23, 38). Once cells are exposed to stressful conditions, such as nutrient starvation, AMPK becomes active and then, reduces the rate of cellular translation through suppression of mTOR activity. Although it was found that AMPK was downregulated and mTOR was upregulated in the patients with AML, a significant positive correlation was observed between these genes both in AML cases and healthy subjects ($p = 0.0002$ and $r = 0.46$ for AML cases and $p = 0.02$ and $r = 0.56$ for healthy subjects). Although the correlation was more significant in AML cases (probably due to the difference between the number of cases in two groups, $n = 60$ for AML cases vs. $n = 30$ for healthy subjects), healthy cells showed a stronger correlation. A similar correlation was also observed between the expression of sestrin 2 and mTOR genes. But, still, there is no knowledge about the mechanisms that are commonly involved in the coordinated regulation of sestrin 2, AMPK and mTOR, at the expression level of genes and thus, further investigations are required to clarify this. A positive correlation was also observed between the expression of AMPK and sestrin 2 genes again in both AML and healthy subjects. As stress-responsible genes share a common promoter and enhancer, this correlation is suggested to be related to common transcription factors generally orchestrating a set of gene expressions (39).

In the present study, a clear disparity was not found in the expression of the investigated genes in terms of different clinical criteria, such as gender, age, AML subtypes, blast count, and specimen type indicating that their expression was irrespective of sex hormones,

cellular senescence, myeloid or monoid origin of AML (AML-M4 and -M5 against other subtypes) differentiation status of cells (AML-M3 against other subtypes), progression of disease at the time of diagnosis and finally, cellular composition of origin source. Our results were congruent with those obtained in the study by Re'cher *et al.*, who showed a constitutive activity of AMPK in all the primary AML cells (37) irrelevant to any criteria emphasizing the importance of genes as a global prognostic factor and therapeutic window in the patients with AML however, more studies are needed using large sample size and clinical trial to further prove these findings. Interestingly, as anticipated, bone marrow and peripheral blood samples tend to express adiponectin similarly. This can be in part due to the eradication of adipocyte niches as a consequence of an accumulation of myeloid progenitor cells in bone marrow space. Although herein, the biopsy was not obtained from the patients to clarify this notion, the percentage of blast cells in the aspirated and peripheral blood samples was a mirror of bone marrow cellular composition.

6. Conclusion

In general, there are both affirmative and negative findings regarding the role of AMPK in AML cells. Some studies have revealed that the patients with AML might benefit from AMPK suppressor agents (40) and others have established that AMPK activator is lethal for AML cells. Based on our results, in a majority of the patients with AML, AMPK probably plays a tumor suppressor role and mTOR has protumor function as confirmed by the expression of genes. Although our results provide a preliminary view regarding the balance of mTOR activity and its negative regulators in the patients with AML, further studies are needed for translation of this scenario from transcriptional level to function of the respective protein, and to determine whether this expression signature could be used as a classifier for the targeted therapy in clinical practices.

Acknowledgments

This work was supported in part by the Shahid Beheshti University of Medical Science, Tehran, Iran, and the Department of Medical Research.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Juliusson G, Antunovic P, Derolf Å, Lehmann S, Möllgård L, Stockelberg D, *et al.* Age and acute myeloid leukemia: real

- world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood*. 2009;**113**(18):4179-4187. doi: 10.1182/blood-2008-07-172007
2. Salarpour F, Goudarzipoor K, Mohammadi MH, Ahmadzadeh A, Faraahi S, Farsani MA. Evaluation of CCAAT/Enhancer Binding Protein (C/EBP) Alpha (CEBPA) and Runt-Related Transcription Factor 1 (RUNX1) Expression in Patients with De Novo Acute Myeloid Leukemia. *Ann Hum Genet*. 2017 Nov;**81**(6):276-283. doi: 10.1111/ahg.12210
 3. Jabari M, Farsani MA, Salari S, Hamidpour M, Amiri V, Mohammadi MH. Hypoxia-Inducible Factor 1-A (HIF1 α) and Vascular Endothelial Growth Factor-A (VEGF-A) Expression in De Novo AML Patients. *Asian Pacific journal of cancer prevention: APJCP*. 2019;**20**(3):705-710. doi: 10.31557/APJCP.2019.20.3.705
 4. Allahbakhshian Farsani M, Kamel M, Mehrpouri M, Heris RS, Hamidpour M, Salari S, Mohamadi MH. The Expression of Interferon Gamma (IFN- γ) and Interleukin 6 (IL6) in Patients with Acute Lymphoblastic Leukemia (ALL). *Pathol Oncol Res*. 2020 Jan;**26**(1):461-466. doi: 10.1007/s12253-018-0536-z. Epub 2018 Nov 15. PMID: 30443842.
 5. Park S, Chapuis N, Tamburini J, Bardet V, Cornillet-Lefebvre P, Willems L, Green A, Mayeux P, Lacombe C, Bouscary D. Role of the PI3K/AKT and mTOR signaling pathways in acute myeloid leukemia. *Haematologica*. 2010;**95**:819-828; doi:10.3324/haematol.2009.013797
 6. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell*. 2017;**168**(6):960-976. doi: 10.1016/j.cell.2017.02.004
 7. Poulain L, Sujobert P, Zylbersztejn F, Barreau S, Stuani L, Lambert M, *et al.* High mTORC1 activity drives glycolysis addiction and sensitivity to G6PD inhibition in acute myeloid leukemia cells. *Leukemia*. 2017 Nov;**31**(11):2326-2335. doi: 10.1038/leu.2017.81. Epub 2017 Mar 10.
 8. Tamburini J, Green AS, Bardet V, Chapuis N, Park S, Willems L, *et al.* Protein synthesis is resistant to rapamycin and constitutes a promising therapeutic target in acute myeloid leukemia. *Blood*. 2009;**114**(8):1618-1627. doi: 10.1182/blood-2008-10-184515.
 9. Christian R, Cădric DS, Cădric D, Bernard P. mTOR, a new therapeutic target in acute myeloid leukemia. *Cell Cycle*. 2005;**4**(11):1540-1549. doi: 10.4161/cc.4.11.2159
 10. Amadori S, Stasi R, Martelli AM, Venditti A, Meloni G, Pane F, *et al.* Temsirolimus, an mTOR inhibitor, in combination with lower-dose clofarabine as salvage therapy for older patients with acute myeloid leukaemia: results of a phase II GIMEMA study (AML-1107). *Br J Haematol*. 2012;**156**(2):205-212. doi: 10.1111/j.1365-2141.2011.08940.x
 11. Perl AE, Kasner MT, Tsai DE, Vogl DT, Loren AW, Schuster SJ, *et al.* A phase I study of the mammalian target of rapamycin inhibitor sirolimus and MEC chemotherapy in relapsed and refractory acute myelogenous leukemia. *Clin Cancer Res*. 2009;**15**(21):6732-6739. doi: 10.1158/1078-0432.CCR-09-0842
 12. Shrestha A, Nepal S, Kim MJ, Chang JH, Kim SH, Jeong GS, *et al.* Critical role of AMPK/FoxO3A axis in globular adiponectin-induced cell cycle arrest and apoptosis in cancer cells. *J Cell Physiol*. 2016;**231**(2):357-369. doi: 10.1002/jcp.25080
 13. Budanov AV, Karin M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. *Cell*. 2008;**134**(3):451-460. doi: 10.1016/j.cell.2008.06.028
 14. Lee JH, Budanov AV, Talukdar S, Park EJ, Park HL, Park H-W, *et al.* Maintenance of metabolic homeostasis by Sestrin2 and Sestrin3. *Cell Metab*. 2012;**16**(3):311-321. doi: 10.1016/j.cmet.2012.08.004
 15. Sanli T, Linher-Melville K, Tsakiridis T, Singh G. Sestrin2 modulates AMPK subunit expression and its response to ionizing radiation in breast cancer cells. *PLoS One*. 2012;**7**(2):e32035. doi: 10.1371/journal.pone.0032035
 16. Sujobert P, Poulain L, Paubelle E, Zylbersztejn F, Grenier A, Lambert M, *et al.* Co-activation of AMPK and mTORC1 induces cytotoxicity in acute myeloid leukemia. *Cell Rep*. 2015;**11**(9):1446-1457. doi: 10.1016/j.celrep.2015.04.063
 17. Green AS, Chapuis N, Maciel TT, Willems L, Lambert M, Arnoult C, *et al.* The LKB1/AMPK signaling pathway has tumor suppressor activity in acute myeloid leukemia through the repression of mTOR-dependent oncogenic mRNA translation. *Blood*. 2010;**116**(20):4262-4273. doi: 10.1182/blood-2010-02-269837
 18. Ma J-J, Shang J, Wang H, Sui J-R, Liu K, Du J-X. Serum adiponectin levels are inversely correlated with leukemia: A meta-analysis. *J Cancer Res Ther*. 2016;**12**(2):897-902. doi: 10.4103/0973-1482.186695
 19. Aref S, Ibrahim L, Azmy E, Al Ashary R. Impact of serum adiponectin and leptin levels in acute leukemia. *Hematology*. 2013;**18**(4):198-203. doi: 10.1179/1607845412Y.0000000059
 20. Chantranupong L, Wolfson RL, Orozco JM, Saxton RA, Scaria SM, Bar-Peled L, Spooner E, Isasa M, Gygi SP, Sabatini DM. The Sestrins interact with GATOR2 to negatively regulate the amino-acid-sensing pathway upstream of mTORC1. *Cell Rep*. 2014;**9**(1):1-8. doi: 10.1016/j.celrep.2014.09.014
 21. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C T method. *Nat Protoc*. 2008;**3**(6):1101-1108. doi: 10.1038/nprot.2008.73
 22. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-Delta Delta C(T))} Method. *Methods*. 2001;**25**(4):402-408. doi:10.1006/meth.2001.1262
 23. Spriggs KA, Bushell M, Willis AE. Translational regulation of gene expression during conditions of cell stress. *Mol Cell*. 2010;**40**(2):228-237. doi: 10.1016/j.molcel.2010.09.028
 24. Mahvi A, Mardani G, Ghasemi-Dehkordi P, Saffari-Chaleshtori J, Hashemzadeh-Chaleshtori M, Allahbakhshian-Farsani M, Abdian N. Effects of phenanthrene and pyrene on cytogenetic stability of human dermal fibroblasts using alkaline comet assay technique. *Proc Natl Acad Sci India Sect B Biol Sci*. 2015;**85**(4):1055-1063. doi: 10.1007/s40011-015-0514-0
 25. Mohamadimaram M, Farsani MA, Mirzaeian A. Evaluation of ATG7 and Light Chain 3 (LC3) Autophagy Genes Expression in AML Patients. *IJPR*. 2019;**18**(2):1060. doi: 10.22037/IJPR.2019.1100682
 26. Hardie DG. AMP-activated protein kinase as a drug target. *Annu Rev Pharmacol Toxicol*. 2007;**47**:185-210. doi: 10.1146/annurev.pharmtox.47.120505.105304. PMID: 16879084.
 27. Liu Y, Beyer A, Aebersold R. On the dependency of cellular protein levels on mRNA abundance. *Cell*. 2016;**165**(3):535-550. doi: 10.1016/j.cell.2016.03.014
 28. Wei JL, Fu ZX, Fang M, Guo JB, Zhao QN, Lu WD, *et al.* Decreased expression of sestrin 2 predicts unfavorable outcome in colorectal cancer. *Oncol Rep*. 2015;**33**(3):1349-1357. doi: 10.3892/or.2014.3701
 29. Pasha M, Eid AH, Eid AA, Gorin Y, Munusamy S. Sestrin2 as a Novel Biomarker and Therapeutic Target for Various Diseases. *Oxid Med Cell Longev*. 2017;2017:3296294.

- doi:10.1155/2017/3296294
30. Chen S, Yan W, Lang W, Yu J, Xu L, Xu X, *et al.* SESN2 correlates with advantageous prognosis in hepatocellular carcinoma. *Diagn Pathol.* 2017;**12**(1):13. doi: 10.1186/s13000-016-0591-2
 31. Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature.* 2009;**460**(7252):259-263. doi: 10.1038/nature08099
 32. Sugiyama M, Takahashi H, Hosono K, Endo H, Kato S, Yoneda K, *et al.* Adiponectin inhibits colorectal cancer cell growth through the AMPK/mTOR pathway. *Int J Oncol.* 2009;**34**(2):339-344. doi: 10.3892/ijo_00000156
 33. Chung SJ, Nagaraju GP, Nagalingam A, Muniraj N, Kuppusamy P, Walker A, Woo J, Györfy B, Gabrielson E, Saxena NK, Sharma D. ADIPOQ/adiponectin induces cytotoxic autophagy in breast cancer cells through STK11/LKB1-mediated activation of the AMPK-ULK1 axis. *Autophagy.* 2017;**13**(8):1386-1403. doi: 10.1080/15548627.2017.1332565
 34. Vousden KH, Ryan KM. p53 and metabolism. *Nat Rev Cancer.* 2009;**9**(10):691. doi: 10.1038/nrc2715
 35. Farsani MA, Rafiee M, Nezhad HA, Salari S, Gharehbaghian A, Mohammadi MH. The Expression of P53, MDM2, c-myc, and P14 ARF Genes in Newly Diagnosed Acute Lymphoblastic Leukemia Patients. *Indian J Hematol Blood Transfus.* 2019;**16**:1-7. doi: 10.1007/s12288-019-01214-6
 36. Quintas-Cardama A, Hu C, Qutub A, Qiu YH, Zhang X, Post SM, *et al.* p53 pathway dysfunction is highly prevalent in acute myeloid leukemia independent of TP53 mutational status. *Leukemia.* 2017;**31**(6):1296-1305. doi: 10.1038/leu.2016.350
 37. Récher C, Beyne-Rauzy O, Demur C, Chicanne G, Dos Santos C, Mansat-De Mas V, *et al.* Antileukemic activity of rapamycin in acute myeloid leukemia. *Blood.* 2005;**105**(6):2527-2534. doi: 10.1182/blood-2004-06-2494
 38. Hindupur SK, González A, Hall MN. The opposing actions of target of rapamycin and AMP-activated protein kinase in cell growth control. *Cold Spring Harb Perspect Biol.* 2015;**7**(8):a019141. doi: 10.1101/cshperspect.a019141
 39. Vihervaara A, Mahat DB, Guertin MJ, Chu T, Danko CG, Lis JT, *et al.* Transcriptional response to stress is pre-wired by promoter and enhancer architecture. *Nat Commun.* 2017;**8**(1):255. doi: 10.1038/s41467-017-00151-0
 40. Saito Y, Chapple RH, Lin A, Kitano A, Nakada D. AMPK protects leukemia-initiating cells in myeloid leukemias from metabolic stress in the bone marrow. *Cell Stem Cell.* 2015;**17**(5):585-596. doi: 10.1016/j.stem.2015.08.019