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Original Article

N6-methyladenosine-modifying enzymes are deregulated in multiple myeloma

Multipl miyelomda N6-metiladenozin modifiye edici enzimlerin düzensizliği

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Abstract

Aim: N6-methyladenosine (m6A) mRNA modification plays essential roles in various cellular processes including regulation of gene expression. However, its role in Multiple Myeloma (MM) biology remains largely unknown. Therefore, the aim of this study is to investigate the expression levels of m6A-regulating enzymes in MM.

Material and Methods: The expression of m6A-regulating enzymes was investigated in monoclonal gammopathy of undetermined significance (MGUS), smouldering MM (SMM), MM and in their normal counterparts using publicly available Gene Expression Omnibus datasets, GSE6477 and GSE47552.

Results: By analyzing GEO DataSets GSE6477 and GSE47552, the expression levels of METTL3, METTL14 and FTO were found to be downregulated in MM, whereas no significant change was found for WTAP, ALKBH5, YTHDF1 and YTHDF2. YTHDF3 expression showed a reduction in MM based on GSE6477 data.

Conclusion: The expression of m6A-modifying genes is deregulated in MM plasma cells compared to normal plasma cells. Altered expression of m6A-modifying genes seems to play a role in the promotion of MM.

Keywords: Multiple myeloma; epitranscriptome; m6A; gene expression; METTL3; FTO

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Öz

Amaç: N6-metiladenozin (m6A) mRNA modifikasyonu, gen ekspresyonunun düzenlenmesi dahil olmak üzere çeşitli hücresel işlemlerde önemli rol oynamaktadır. Bununla birlikte, Multipl Miyelom (MM) biyolojisindeki rolü büyük ölçüde bilinmemektedir. Bu nedenle, bu çalışmanın amacı MM'da m6A düzenleyici enzimlerin ekspresyon düzeylerini araştırmaktır.

Gereç ve Yöntemler: M6A düzenleyici enzimlerin ekspresyonu kamuya açık Gene Expression Omnibus veri kümeleri GSE6477 ve GSE47552 kullanılarak, anlamı belirsiz monoklonal gamopati (MGUS), smouldering MM (SMM), MM ve normal muadillerinde araştırılmıştır.

Bulgular: GEO data setleri GSE6477 ve GSE47552 analiz edilerek, METTL3, METTL14 ve FTO ekspresyon seviyelerinin MM'da downregüle olduğu bulunurken WTAP, ALKBH5, YTHDF1 ve YTHDF2 için anlamlı bir değişiklik bulunmadı. YTHDF3 ifadesi, GSE6477 verisine dayalı olarak MM'de bir azalma gösterdi.

Sonuç: M6A değiştirici genlerin ifadesi, normal plazma hücrelerine kıyasla MM plazma hücrelerinde deregüle edildiği bulunmuştur. M6A'nın modifiye edici genlerinin değişmiş ifadesi, MM'nın oluşumunda rol oynar gibi görünmektedir.

Anahtar kelimeler: Multipl miyelom; epitranskriptom; m6A; gen ekpresyonu; METTL3; FTO

Introduction

Multiple Myeloma (MM) arises from the proliferation of abnormal plasma cells that secrete monoclonal antibodies in the bone marrow and accounts for approximately 15% of all hematologic malignancies (1). This malign disease emerges from a pre-malignant stage termed monoclonal gammopathy of undetermined significance (MGUS) and more advanced pre-malignant stage termed smouldering (or indolent) MM (SMM) (2). The progression from MGUS and SMM to MM is determined by the presence of different amounts of serum monoclonal proteins and the symptomps of hypercalcemia, renal failure, anaemia, and bone destruction (2,3). Despite the dramatic improvements in the treatment of MM including the introduction of proteasome inhibitors, immunomodulatory agents and monoclonal antibodies, MM remains still incurable due to many patients inevitably becoming refractory to the current treatments. Therefore, a better understanding of genomic, epigenomic and proteomic pathways in this disease is needed to define new therapeutic targets, specifically to evaluate the various agents targeting tumor cells and tumor microenvironment, and to overcome the development of resistance to antimyeloma agents.

TThe recent insights into the regulation of gene expression have had a prominent impact on the molecular mechanisms underlying the biology of MM. Gene expression is mainly adjusted by transcription factors and then post-transcriptional modifications. Post-transcriptional modifications including direct chemical modification, editing or nontemplated nucleotide additions have opened a new field of 'epitranscriptome' which involves RNA maturation, stability, transportation, and yield of the protein translation (4). Although all cellular RNAs from all living organisms can be post-transcriptionally modified by more than 100 distinct chemical modifications (5), major mRNA modifications in eukaryotic transcriptome consist of N6-methyladenosine, N6,2'-O-dimethyladenosine, 5-methylcytidine, 5-hydroxylmethylcytidine, inosine, pseudouridine and N1-methyladenosine (6). Among these modifications the methylation of adenines at the N6 position called N6-methyladenosine (m6A) is the most abundant modification. m6A can change the secondary structure of the mRNA that leads to an alteration in its splicing, translation, and cytoplasmic degradation (7).

m6A has been reported to be a more extent in 5' untranslated regions (UTRs) and near stop codons in the 3' end of the mRNA, while a lesser extent in introns and long internal exons (7-9). The recent mapping of m6A sites throughout the transcriptome of human cells by high-throughput sequencing revealed that 118000 m6A peaks are found in over 12000 genes (4,10). m6A modification can vary between cell types and be dynamically regulated by several highly conserved enzymes. These enzymes are the m6A 'writers' which attach methyl to the specific adenines on the target RNAs, 'erasers' which reversibly removes the methyl group from m6A and 'readers' that regulate the fate of modified mRNAs by binding to m6A.

m6A 'writers' in mammalian cells include a methyltransferase complex with both catalytic and regulatory units including METTL3 (methyltransferaselike 3) shown to be the main methyltransferase responsible for m6A modification (11);

METTL14 the other catalytic subunit, and WTAP (Wilms tumor 1 associated protein) the controller of the process (12,13). m6A 'erasers' consist 2 demethylases, fat mass and obesity-associated (FTO) and alkylated DNA repair protein alkB homolog 5 (ALKBH5). The best characterized of m6A 'readers' are the YTH domain-containing proteins, YTHDF1, YTHDF2, and YTHDF3 that selectively bind m6A-modified RNAs (14,15).

The identification of the contributors of m6A modification and the development meRIP-sequencing (m6A-seq) technology shed a light into defining the roles of m6A mRNA modification in cancer biology. The involvement of the writers, readers, and erasers of m6A modification in the promotion of tumorigenesis was reported by many recent studies (16). However, the functional roles of the contributors of m6A modification in MM progression need to be determined. Therefore, the aim of this study is to examine the expression levels of the writers, readers, and erasers of m6A modification using MM patient datasets GSE6477 and GSE47552 (17,18).

Materials and Methods

Publicly available Gene Expression Omnibus datasets (https://www.ncbi.nlm.nih.gov/geo/; accession numbers GSE6477 and GSE47552) (17,18) were analyzed using GEO2R **(http://www.ncbi.nlm.nih.gov/geo/geo2r/)** to study gene expression of the contributors of m6A modification which are METTL3, METTL14, WTAP, FTO, ALKBH5, YTHDF1, YTHDF2, and YTHDF3 in plasma cells derived from MGUS, SMM and MM patients and healthy subjects. GEO datasets [GSE6477 (Normal plasma cells (NPC): N = 15; Monoclonal gammopathy of undetermined significance (MGUS): N = 21; smoldering MM (SMM): N = 24; newly-diagnosed MM (NDMM): N = 69; relapsed/refractory MM (RRMM): N = 55) and GSE47552 (NPC: N = 5; MGUS: N = 20; SMM: N = 33; MM: N = 41) were analyzed using the gene chips of Affymetrix Human Genome U133 Plus 2.0 Array and Affymetrix Human Gene 1.0 ST Array [transcript (gene) version], respectively. The probe IDs for each transcript on the Affymetrix Human Genome U133A Array and Affymetrix Human Gene 1.0 ST Array were displayed in Table 1. Default settings were applied when using GEO2R website and the results were obtained by using the function 'Save all results' and were further analyzed. Fold change obtained in the normal plasma cells (NPCs) was set to 1. Statistical analysis was performed with one-way Anova Dunnett's multiple comparisons test using GraphPad Prism 6.0. This study was based on data analysis of GEO datasets GSE6477 and GSE47552 using GEO2R. All the experimental conditions in these datasets were approved by the research ethic committees. Therefore, this study did not involve ethics approval and consent to participate.

Table 1. Probe IDs for each gene on the Affymetrix Human Genome U133A Array and Affymetrix Human Gene 1.0 ST Array

Gene Symbol	Probe ID for the Affymetrix Human Genome U133A Array (Platform GPL96)	Probe ID for the Affymetrix Human Gene 1.0 ST Array [transcript (gene) version] (Platform GPL6244)
METTL3	209265_s_at	7977749
METTL14	-	8097066
WTAP	203137_at	8123129
FTO	209702_at	7995655
ALKBH5	-	8005399
YTHDF1	221741_s_at	8067593
YTHDF2	217812_at	7899519
YTHDF3	221749_at	8146637

Results

Gene expression levels of m6A-metabolizing enzymes were analyzed in plasma cells derived from MGUS, SMM and MM patients as well as in their normal counterparts using GEO datasets, GSE6477 (17) and GSE47552 (18). METTL3 the main m6A writer was found to be significantly downregulated in MM plasma cells compared to NPCs by the analysis of two datasets (17,18) (Figure 1).

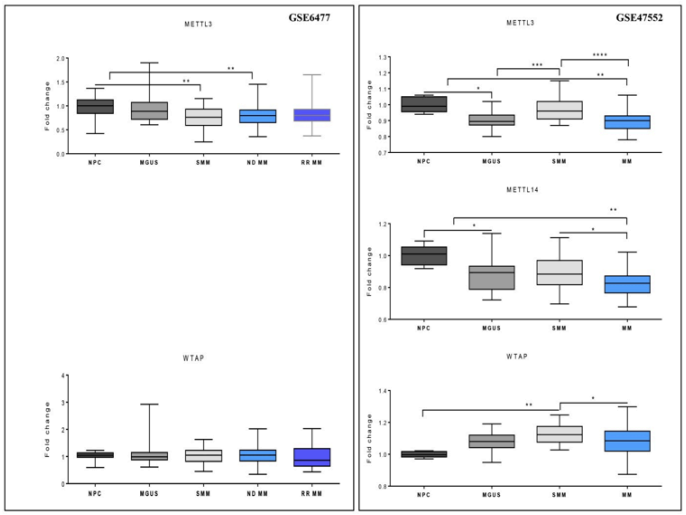

Figure 1. *METTL3 and METTL14 expressions are downregulated in MM. By analyzing GEO DataSets GSE6477 and GSE47552, expression levels of individual m6A writers (i.e., METTL3, METTL14, and WTAP) illustrated by Box-whisker plots were determined in MM. NPC: Normal Plasma Cells (n=15 in GSE6477; n=5 in GSE47552); MGUS: Monoclonal Gammopathy of Undetermined Significance (n=21 in GSE6477; n=20 in GSE47552); SMM: Smoldering Multiple Myeloma (n=24 in GSE6477; n=33 in GSE47552). In GSE47552 study, MM stands for Multiple* Myeloma (n=41); In GSE6477

*study, ND MM stands for Newly Diagosed Multiple Myeloma, (n=69) and RR MM stands for Relapsed/Refractory Multiple Myeloma, (n=27). One-way Anova Dunnett's multiple comparisons test was performed used to determine the significance. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.*

Furthermore, analysis of GSE6477 dataset showed a decrease in the expression of METTL3 in SMM plasma cells compared to NPCs, while the same result was not observed by the interpretation of GSE47552. Analysis of GSE6477 dataset revealed that METTL3 expression is higher in SMM cells compared to those derived from MGUS and MM patients (Figure 1). METTL14 the other m6A writer was also significantly decreased in both MGUS and MM plasma cells compared to normal counterpart by the analysis of GSE47552, whereas METTL14 could not be found in the list of genes in GSE6477 (Figure 1). The expression of WTAP the stabilizer of the writing complex was found to be similar between NPCs and MGUS, SMM and MM cells by the interpretation of the dataset GSE6477 (Figure 1). However, WTAP expression was notably overexpressed in SMM plasma cells but not in MM plasma cells compared to NPCs and a decrease in WTAP expression between SMM and MM cells was found to be significant by the analysis of GSE47552 (Figure 1).

m6A demethylase FTO was found to be significantly downregulated in MM cells compared to normal NPCs by the analysis of two datasets (17,18) (Figure 2).

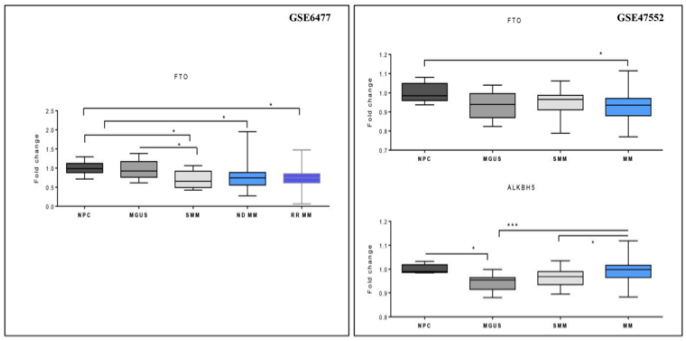

Figure 2. *Decreased expression of FTO in MM. Expression of m6A erasers FTO and ALKBH5 was analyzed using GSE6477 and GSE47552. Compared to NPCs, the expression of FTO was decreased in MM, whereas no significant change was found for ALKBH5. NPC: Normal Plasma Cells (n=15 in GSE6477; n=5 in GSE47552); MGUS: Monoclonal Gammopathy of Undetermined Significance (n=21 in GSE6477; n=20 in GSE47552); SMM: Smoldering Multiple Myeloma (n=24 in GSE6477; n=33 in GSE47552). In GSE47552 Dataset, MM: Multiple Myeloma (n=41); In GSE6477 Dataset, ND MM: Newly Diagosed MM, (n=69); RR MM: Relapsed/Refractory MM, (n=27). *p < 0.05; **p < 0.01; ***p < 0.00; One-way Anova.*

Analysis of GSE47552 dataset showed that the expression of another m6A

demethylase ALKBH5 is comparable between NPCs and MM cells. Furthermore, a significant decrease in the expression of ALKBH5 was only observed in MGUS cells compared to NPCs. Of note, ALKBH5 could not be found in the list of genes in GSE6477.

The expression of YTHDF1 and YTHDF2 which are the first two of methyl readers was not altered in MM cells compared to NPCs by the analysis of two datasets (17,18) (Figure 3).

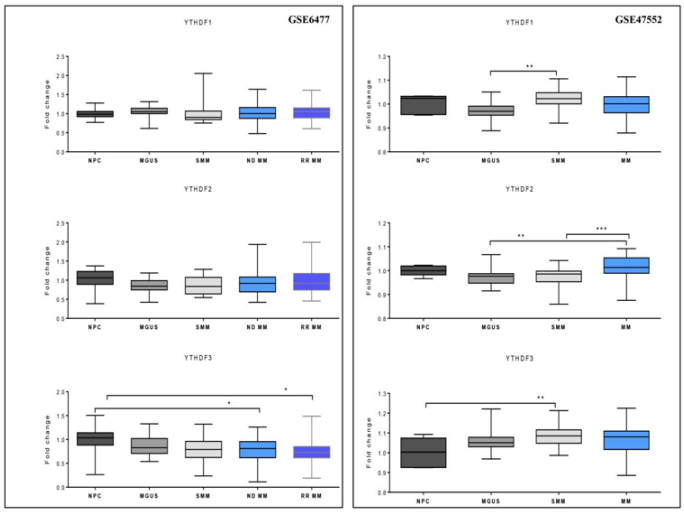

Figure 3. *Altered expression of YTHDF3 in MM. m6A readers YTHDF1 and YTHDF2 expressions were similar between NPCs and MM, while YTHDF3 expression was deregulated in MM. NPC: Normal Plasma Cells (n=15 in GSE6477; n=5 in GSE47552); MGUS: Monoclonal Gammopathy of Undetermined Significance (n=21 in GSE6477; n=20 in GSE47552); SMM: Smoldering Multiple Myeloma (n=24 in GSE6477; n=33 in GSE47552). In GSE47552 Dataset; MM: Multiple Myeloma (n=41); In GSE6477 Dataset; ND MM:*

Compared to NPCs, the expression of YTHDF3 the third one of the methyl readers was significantly reduced in both newly diagnosed (ND) and relapsed/refractory (RR) MM cells according to the analysis of GSE6477. However, YTHDF3 expression was similar between NPCs and MM cells by the interpretation of GSE47552.

Thus, the expression of m6A methyltransferases METTL3 and METTL14 was significantly decreased, whereas WTAP expression was comparable in MM cells. m6A demethylase FTO was also significantly reduced in MM. However, the expressions of ALKBH5, YTHDF1 and YTHDF2 were found to be similar between NPCs and MM cells. When these two datasets were compared for YTHDF3 expression, the result found to be contradictory. The results were summarized in Table 2.

Table 2. m6A-regulating enzymes expression in MGUS, SMM and MM compared to NPCs using publicly available GSE6477 and GSE47552

Gene expression level compared to NPCs							
Protein	Function	Dataset	MGUS	SMM	ND MM	RR MM	MM
METTL3	m6A writer	GSE6477	No change	Decrease	Decrease	No change	-
		GSE47552	Decrease	No change	-	-	Decrease
METTL14	m6A writer	GSE6477	-	-	-	-	-
		GSE47552	Decrease	No change	-	-	Decrease
WTAP	m6A writer	GSE6477	No change	No change	No change	No change	No change
		GSE47552	No change	Decrease	-	-	No change
FTO	m6A eraser	GSE6477	No change	Decrease	Decrease	Decrease	-
		GSE47552	No change	No change	-	-	Decrease
ALKBH5	m6A eraser	GSE6477	-	-	-	-	-
		GSE47552	Decrease	No change	-	-	No change
YTHDF1	m6A reader	GSE6477	No change	No change	No change	No change	-
		GSE47552	No change	No change	-	-	No change
YTHDF2	m6A reader	GSE6477	No change	No change	No change	No change	-
		GSE47552	No change	No change	-	-	No change
YTHDF3	m6A reader	GSE6477	No change	No change	Decrease	Decrease	-
		GSE47552	No change	Decrease	-	-	No change

Discussion

By analyzing publically available GEO datasets, this study shows that the expressions of METTL3 and METTL14 m6A methyltransferases and m6A demethylase FTO were decreased in MM cells. Furthermore, the expression of ALKBH5, YTHDF1 and YTHDF2 were not significantly changed in MM cells, while based on the analysis of GSE6477 data, YTHDF3 expression was found to be reduced in MM cells compared to NPCs.

m6A mRNA methylation plays essential roles in various bioprocesses such as cell proliferation and differentiation (19). Study of m6A mRNA modification and its regulation in cancer biology has attracted great interest in recent years and showed its important role in cancer initiation and progression (16). The m6A methylase complex termed as m6A 'writers' has mainly consisted of METTL3, METTL14 and WTAP. The m6A demethylases called m6A 'erasers' consist FTO and ALKBH5. The best characterized of m6A 'readers' are YTHDF1, YTHDF2, and YTHDF3 that selectively bind m6A-modified RNAs (12-16).

To my knowledge, for the first time, this study uncovered the expression levels of m6A mRNA modifying enzymes in MM biology. This study displayed an important decrease in METTL3 and METTL14 expressions in MM, suggesting a role in MM development. METTL3 was reported to be an important controller of human myeloid differentiation and its deletion in myeloid leukemia cells led to an increase in cell differentiation and apoptosis (20). METTL14 likewise METTL3 is downregulated during myeloid differentiation and eminently expressed in AML cells which have t(11q23), t(15;17), or t(8;21) (20). Knockdown of METTL3 or METTL14 induced an alteration in mRNA m6A enrichment and changed mRNA expression of genes that lead to promote glioblastoma stem cell (GSC) growth and tumorigenesis (21). Bansal and colleagues reported that WTAP expression was increased in AML patients and knockdown of WTAP in a leukemia cell line led to a reduced proliferation, increased differentiation, and elevated apoptosis (22). However, in this study the expression of WTAP was found to be similar between NPCs and MM.

Increased expression of FTO is related to the progression of various cancers, including AML cells carrying t(11q23)/MLL, t(15;17)/PML-RARA and FLT3-ITD (23). In addition, ALKBH5, an another m6A demethylase, was found to demethylate forkhead box protein M1 (FOXM1) nascent transcripts and be highly expressed in GSCs (24). ALKBH5. However, in this study FTO expression was found to be notably decreased in MM plasma cells compared to NPCs, while ALKBH5 expression was found unchanged in MM cells.

This study also showed that expression levels of YTHDF1 and YTHDF2, m6A "reader" proteins, were not significantly altered in MM plasma cells compared to NPCs. However, Chen et al. showed that human pancreatic cancer cells have increased expression of YTHDF2 that plays an important role in proliferation and epithelial-mesenchymal transition (EMT) (25). YTHDF3 expression was found to be decreased in plasma cells derived from both ND and RR MM patients compared to their normal counterparts. Although, the current study revealed a decrease expression in METTL3, METTL14, FTO and YTHDF3, these m6A-modifying genes appear to have no effect on MM progression. In summary, decreased m6A methylation seem to play a role in MM development.

Conclusion

The analysis reported here shows that the expression of m6A-modifying genes is deregulated in MM plasma cells compared to NPCs. Altered expression of m6A-modifying genes appears to play a role in the development of MM. Further investigation to determine the level of m6A modification on mRNAs in MM cells is required.

Declaration of conflict of interest

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

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