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# Circulating tumour cells differentially express upregulated cancer biomarkers "lncRNAs and miRNAs" compared to bone marrow biopsy samples in multiple myeloma patients

**Gizem AYNA DURAN***

Department of Biomedical Engineering, Faculty of Engineering, Izmir University of Economics, İzmir, Türkiye

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**Abstract**

Easy-to-apply liquid biopsy technique and detection of differentially expressed miRNA/lncRNAs (DEGs) may be more beneficial in multiple myeloma (MM) compared to bone marrow (BM) biopsy. We compared the gene expression levels of circulating tumor cells and BM cells in MM patients and showed differentially upregulated circulating tumor cell-derived miRNAs and lncRNAs. DEGs and related biological pathways were identified by using the R-LIMMA package, ShinyGO 0.77, and LncSEA2.0 tools. Three hundred nine lncRNAs/16 miRNAs were detected as differentially upregulated in MM patients' circulating tumour cells. Among them, miRNAs (mainly has-miR-103a) and lncRNAs (MEG3, NEAT1, PCAT1) were detected, and only a few miRNAs and lncRNAs were related to MM in a limited number of studies. Drugs that interact with these lncRNAs were also identified. The fact that miRNAs/lncRNAs related to MM are also detected in tumor circulating cells indicates that a technically easier liquid biopsy may verify and even replace BM biopsy.

**Keywords:** Circulating tumour cells, multiple myeloma, DEGs, miRNA, lncRNAs

## 1. Introduction

Multiple myeloma (MM) is a type of hematological cancer that is fatal and can adversely affect survival in a short time. MM is known as the second most common hematological malignancy after non-Hodgkin lymphoma, and it is observed at a rate of 17.1% among blood cancers and 1.2% of all cancers (1,2). Since 1990, the incidence of multiple myeloma, a fatal hematologic cancer, has increased by 126% globally. In addition, it has been determined that this increase is over 40% in the USA. In addition, the global death rate from MM is increasing by 94% (3). More effective diagnosis and treatment strategies are still to be optimized to increase the survival rates and decrease the deaths in MM.

MM is a genetically heterogenous type of cancer characterized primarily by the clonal growth and accumulation of neoplastic plasma cells (PCs; B lymphocytes) in the bone marrow (BM). Cancerous plasma cells accumulating in the later stages of the disease pass from the bone marrow to the peripheral blood and gradually spread to different parts of the body. Considering the spread of the disease, diagnostic methods accepted and actively used in clinical practice were developed by the International Myeloma Working Group (IMWG) (4,5). The diagnosis of MM can be based on the symptoms observed in patients and common pathologies (myeloma defining events (MDE)), as well as a widely used method (such as CRAB features; bone lesions, hypercalcaemia, anaemia, and renal failure) with bone marrow aspiration and biopsy in PCs. It is the detection of changes at the genetic level (mutations such as trisomies, translocations and deletions, expression levels). These genetic changes include 29 high-risk markers identified by 17 clinical validation studies to date (1,2).

Although BM aspirates and biopsy play an important role in the diagnosis of MM, it is a diagnostic method that is not easy to apply because it is an invasive procedure that commonly increases the anxiety level of patients. Furthermore, since MM is a heterogeneous disease at the cytogenetic level and in the accumulation of genetic mutations, it may sometimes be impossible to detect subclone cancer cells by BM aspirate and biopsy collected from a single site. Therefore, alternative diagnostic methods are currently the focus of the research world. One of these non-invasive methods is the analysis of cell-free DNA and circulating tumor cells (CTCs), also known as "liquid biopsy". Although there is no specific standardized procedure, liquid biopsy has become a technique with advantages because it is painless, easily reproducible, can determine tumor heterogeneity, and validate most genetic aberrations from bone marrow biopsy (6, 7).

*Correspondence: gizem.duran@ieu.edu.tr

Multiple myeloma is a type of cancer suitable for study in terms of determining the routes cancer cells move and explaining their metastatic properties. Although the primary tumor in MM originates from the bone marrow, the cancerous cells are observed to pass into the peripheral blood after a while. These cells in circulation spread throughout the body to create micrometastatic (MGUS) and multiple lytic lesions, respectively (8). For example, as shown in studies on CTCs in the diagnosis of MM, there are studies showing that there are CTCs in the peripheral blood at many stages of MM, such as newly diagnosed MM, relapsed MM, and monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (9, 10, 11). Many years of studies are required to make the CTC analysis the gold standard in MM, but studies so far have shown that it is a method that can make important contributions to the diagnosis and especially to the determination of the metastasis level of the disease.

It has been shown that non-coding RNAs (ncRNAs) such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circulating RNAs (circRNAs) play a role in MM development and progression (metastasis, drug resistance and proliferation) (12). miRNAs are 19-22 nucleotides, and lncRNAs are non-coding RNA sequences of 200 nucleotides. Both miRNAs and lncRNAs and their aberrations have been shown to be important in the biological pathways involved in the development of MM cancer. For example, miRNAs play important roles in processes such as methylation patterns, drug resistance, prognosis, microenvironment formation, and immune regulation in MM (13). lncRNAs, on the other hand, play a role in processes such as epigenetic reprogramming mechanisms, development of resistance to cell death, inflammation triggered by tumour formation, bone metastasis, and plasticity (14). In addition, studies have shown that the interactions of lncRNAs with miRNAs play a role in MM (12).

In our study, we compared the gene expression levels of patients with newly diagnosed MM with gene intensity data obtained from circulating tumor cells and those of patients with MM diagnosed by bone marrow biopsy, and we showed differentially upregulated miRNA and lncRNA in circulating tumor cells. The relationship of most of the miRNAs and lncRNAs we obtained in the results of our study with MM has not been mentioned in the literature yet, and those shown to be associated with MM have been investigated in a limited number of studies.

## 2. Materials and Methods

### 2.1. Data Acquisition and Data Processing

A total of 1046 multiple myeloma (MM) patients were included in this study. Microarray-based gene intensity values of MM patients' genes were used from the Gene Expression Omnibus (GEO) database [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array (GPL570) platform with 54,697 probes. Out of 1046 patients, 109 were newly diagnosed MM patients in the context of the HOVON131 clinical trial (GSE164701) (will be named CC patients). Gene intensity values of these 109 MM patients were obtained from circulating tumor cells, and MM cells were enriched using CD138-specific beads (15). Furthermore, 937 newly diagnosed MM patients' gene expression profiles were determined by using their bone marrow samples (GSE24080 dataset from MAQC-II Project, GSE2658) (will be named as BM-1 and BM-2 respectively) (16). Additionally, gene intensity values of 22 healthy individuals (from GSE5900) were included in the data to determine differentially expressed genes (DEGs). Raw data of GSE 24080, GSE164701 and GSE5900 (22 healthy control) as CEL files were used and "Robust Multi-Array Average" (RMA) normalization was achieved by using the Bioconductor R program. Furthermore, CEL files of GSE2658 data could not be obtained, and log2 transformation was applied to the data via GEO2R analysis.

### 2.2. Statistical Analysis

Genes that differ between healthy samples with and without MM cancer were identified using the linear modeling method included in the R LIMMA (version 4.2.2) package separately for all datasets. The p-value of $<0.05$ was accepted as the threshold value for deciding on statistical significance. The results of this analysis are also visualized with Volcano plots. Following DEG analyses, upregulated and downregulated genes were determined in patients in the circulating tumor cell dataset (GSE 164701), which were different from the genes obtained from the other two datasets (GSE24080, GSE2658).

### 2.3. Gene Enrichment Analysis

Furthermore, LncSEA2.0 web-based tool was used to clarify the cancer type and pathways affected by lncRNAs (https://bio.liclab.net/LncSEAv2/analysis/gene_set_enrichment.php) by choosing "Cancer Hallmark" and "Disease-Lnc2Cancer" options. ShinyGO 0.77 was used to show the types of genes (protein coding, lncRNA or miRNA) detected as upregulated in CC patients. Furthermore, an upregulated mRNAs-miRNAs list was obtained using ShinyGO 0.77 miRNA.Target.miRTarBase option. On the other hand, drugs targeting key lncRNAs determined in our study were identified using the LncSEA2.0 web tool.

## 3. Results

### 3.1. Detection of differentially expressed genes in circulating tumor cells in MM patients

In our study, microarray gene intensity data of a total of 1046 patients diagnosed with multiple myeloma were used, and the Volcano graphs showed that there were many significant up- and down-regulated genes in all three datasets (Fig. 1A, B, C). While 21972 genes were down-regulated in patients diagnosed via circulating tumor cell analysis (CC) compared to healthy individuals, 23374 and 26727 genes were found to be down-regulated in BM-1 and BM-2 patient groups, respectively. In addition, 14921 and 8402 genes were upregulated in BM-1 and BM-2 patient groups, respectively, when compared with healthy individuals, while this number was determined as 22322 genes in the CC patient group (Table 1).

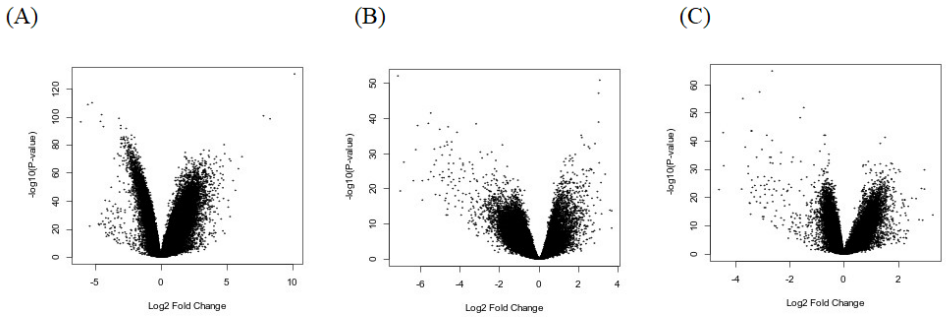


**Fig. 1.** Volcano plots of circulating cells derived differentially expressed genes (DEGs) in MM patients; Volcano plots were generated by DEG analysis in R program respectively for (A) CC patient group, (B) BM-1 patient group, and (C) BM-2 patient group

**Table 1.** Datasets and related information used in the study

| Circulating/Tissue | Circulating Tumour Cells (CC) | Plasma Cells from Bone Marrow (BM-1) | Plasma Cells from Bone Marrow (BM-2) |
|---|---|---|---|
| NCBI GEO Database (GSEs) | GSE164701 | GSE24080 | GSE2658 |
| Number of Control (from GSE5900) | 22 | 22 | 22 |
| Number of Patients | 109 | 559 | 378 |
| Number of Genes Analysed | 54675 | 54675 | 54675 |
| Down-regulated Protein Coding Genes | 21972 | 23374 | 26727 |
| Up-regulated Protein Coding Genes | 22322 | 14921 | 8402 |
| Non-Significant Genes | 10381 | 16380 | 19546 |

As can be seen in Table 1, the study focused only on the upregulated genes, as there were many more and different genes upregulated in the CC patient group compared to the other patient groups. Upon detecting the genes that were upregulated differently from healthy individuals in each dataset, as a next step, genes that were upregulated in patients in the CC group, unlike the other BM-1 and BM-2 groups, were determined. According to our results, a total of 5363 genes were shown to be upregulated in CC patients, unlike other patients, and these genes were mostly found to be protein-coding genes, lncRNAs and miRNAs (Fig. 2 A, B).

Our study determined that 4841 of these genes were upregulated protein coding genes, 16 of them were upregulated miRNAs, and 309 were upregulated lncRNAs. Among the remaining genes, there were genes such as polymorphic and processed pseudogenes, miscRNAs (RN7SL731P, Y_RNA), scaRNAs (SCARNA13), snoRNAs (SNORD89, SNORD63B, SNORD27, SNORA31, SNORD118, SNORD3B-1, SNORA21B, SNORA37) and snRNAs (RNU1-129P, RNU4-78P, RNU1-70P, RNU6-703P, RNU6-195P).

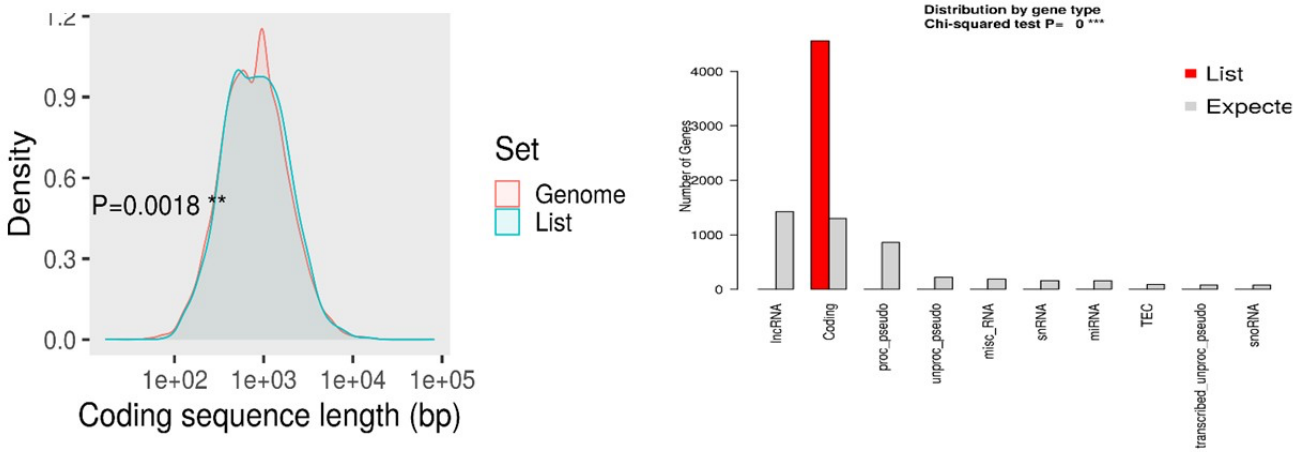


**Fig. 2.** Up-Regulated Circulating Cells Derived Circulating DEGs are mostly protein-coding genes, miRNAs, and lncRNAs; (A) The density plot obtained from ShinyGO 0.77 compared to whole genome, (B) the bar graph of the gene distributions determined as upregulated in CC patient group

### 3.2. Differentially expressed miRNAs in circulating tumor cells of MM patients

Differently expressed and upregulated circulating miRNAs in MM patients, the relationship of these detected 16 miRNAs with different types of cancers and molecular mechanisms has been summarized according to the findings of the updated literature (Table 2). Furthermore, a few circulating miRNAs such as has-miR-429, has-miR-210, has-miR-15a, has-miR-1180, and has-miR-103a-2 were related to multiple myeloma in a limited number of studies (17, 18, 19). Some other miRNAs have not yet been related to MM.

**Table 2**. Differently Expressed and Up-regulated Circulating miRNAs in MM patients

| Up-regulated Circulating miRNAs | Molecular Mechanism(s) in Cancer | Related Cancer(s) | Reference |
|---|---|---|---|
| | Proliferation | Non-small cell lung cancer | (20) |
| **miR-429** | Chemoresistance | Ovarian Cancer | (21) |
| | **Invasion and Migration** | **Multiple Myeloma** | (17) |
| | Proliferation and migration | Breast Cancer | (22) |
| miR-4775 | invasion and metastasis | Colorectal Cancer | (23) |
| miR-6829 | proliferation | Bladder cancer | (24) |
| miR-548n | Metastasis | endometrial endometrioid carcinoma | (25) |
| miR-4680 | Progression | Glioma | (26) |
| **miR-210** | Hypoxia | head and neck cancer | (27) |
| | **Hypoxia** | **Multiple myeloma** | (18) |
| | Angiogenesis | Non-small cell lung cancer | (28) |
| **miR-15a** | **cell growth suppression and apoptosis** | **Multiple myeloma** | (29) |
| | **Tumour proliferation** | **Multiple myeloma** | (40) |
| | cell proliferation and migration (hsa-miR-15a-5p) | Colorectal carcinoma | (30) |
| miR-6883 | Proliferation (hsa-miR-6883-5p) | Colorectal cancer | (31) |
| **miR-1180** | proliferation and malignancy (miR-1180-3p) | Hepatocellular carcinoma | (32) |
| | **Tumor suppression** | **Multiple myeloma** | (19) |
| miR-133a-1 | tumorigenesis, progression, autophagy, and drug-resistance | Osteosarcoma, esophageal cancer (EC), colorectal cancer (CRC), non-small cell lung cancer (NSCLC), bladder cancer, breast cancer and gastric cancer | Reviewed in (33) |
| miR-4751 | Drug resistance | non-small cell lung cancer | (34) |
| miR-372 | suppresses tumour proliferation and invasion | renal cell carcinoma | (35) |
| **miR-103a-2** | **Drug resistance(miR-103a-2-5p)** | **Multiple myeloma** | (36) |
| miR-3618 | Drug resistance | non-small cell lung cancer | (34) |
| miR-6881 | P53-Mediated Competing Endogenous RNA Network (hsa-miR-6881-3p) | Hepatocellular carcinoma | (37) |
| miR-934 | cell proliferation, migration, invasion, and angiogenesis | colorectal cancer | (38) |

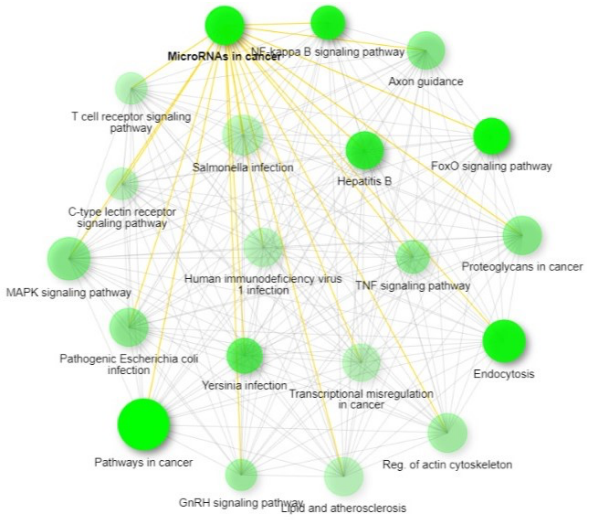


**Fig. 3.** Up-regulated protein coding genes are related to biological pathways including miRNA 4841 upregulated protein coding genes are enriched and miRNA interaction has been shown in ShinyGO KEGG pathway

In our study, according to the ShinyGO 0.77 webtool network analysis, it was shown that 4841 genes, different from BM-1 and BM-2 patients, play a role in different biological pathways in CC patients. These pathways also have interactions with miRNAs (Fig. 3). Furthermore, unlike BM-1 and BM-2 patients, 174 of 4841 upregulated protein-coding genes were associated with Hsa-miR-103a-3p (Table 3). These results were obtained using the miRNA.Target.miRTarBase option of the ShinyGO 0.77 web tool.

### 3.3. Differentially expressed lncRNAs in circulating tumor cells of MM patients

Similarly, related cancers of upregulated lncRNAs are shown in Fig. 2. According to our results, circulating upregulated lncRNAs were not related to multiple myeloma in Fig. 4 since the graph showed the first 14 cancer types of 41 related to our lncRNA gene set (Table 4). The first two types of cancer associated with the highest number of genes and cancers associated with the least number of genes (including MM) are presented in Table 4. According to the lncRNA cancer

interaction analysis using the LncSEA online tool, multiple myeloma was presented as the 41st cancer type at the end of the list. According to the results of the analysis, multiple myeloma was associated with 3 of the lncRNAs and these lncRNAs are maternally expressed gene 3 (MEG3), nuclear paraspeckle assembly transcript 1 (NEAT1), prostate cancer-associated transcript-1 (PCAT-1). Furthermore, in our study, the circulating and differentially upregulated lncRNAs in MM patients were also associated with cancer pathways, as summarized in Table 5.

According to LNCSEA2.0 webtool results, candidate drugs targeting MEG3, NEAT1, and PCAT1 lncRNAs have been identified (Table 6).

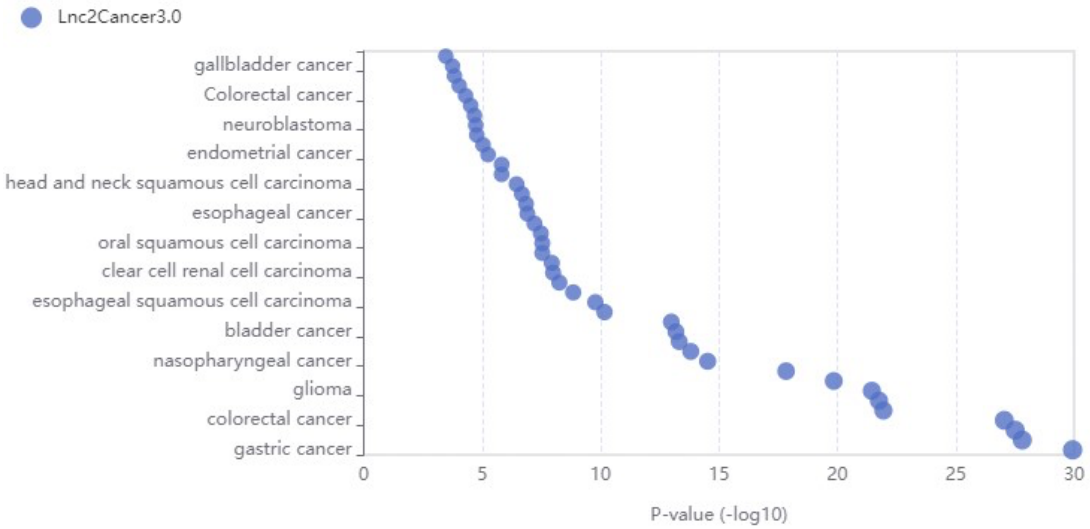


**Fig. 4.** Up-regulated and circulating cells derived lncRNAs in CC patients lncRNAs were related to 41 cancer types. Only 14 cancer types are indicated in the figure.

**Table 3.** Hsa-miR-103a-3p is related to 174 upregulated protein coding genes in CC patients

| Enrichment FDR | nGenes | Pathway Genes | Fold Enrichment | Pathway | Genes |
|---|---|---|---|---|---|
| 2.14E-17 | 174 | 453 | 1.919771864 | Hsa-miR-103a-3p target gene | ENPP4 LASP1 CYP26B1 POLDIP2 LAMP2 ALDH3B1 VCAN MAP4 CDK17 SLC2A3 GNAI3 NUCKS1 TPD52 PPP2R5C RIF1 EXD2 POMGNT1 FKBP1A NUP50 IFT74 SF3A1 CARD10 TNRC6B NIN DICER1 PPP1R16B RNMT CDADC1 N4BP1 CDK6 USP42 TLE4 CPEB3 LARP4B SHOC2 TBC1D12 MED13 PRDM4 CAMKK2 GSG1 HCFC2 HDDC2 PDE4D PRKAR2A GNAT1 KLHL18 ID2 KCNC4 C1orf21 CREB1 FCF1 FAM98A CD274 EPC1 RBBP6 ODF2L HNRNPA2B1 RECK CDK2 RUNX2 TMEM255A GGA3 PPP1R12C GPCPD1 AGO3 ATG14 DYRK2 FGFRL1 YWHAH HOXD13 MPDU1 CCNT1 MIS18BP1 TULP4 STK33 CLIP1 LATS1 SH3BP5 PNISR NUMB ARGLU1 MAP3K7 USP15 GNS STX6 CAB39 KLF4 CPEB2 SDCBP ACTR2 ARL3 PPIG FGF2 GABARAPL1 SETD1B CUL4A ZFHX3 ZCCHC14 ABL2 ARL8A EML4 PIK3R1 ATG12 RNF217 DOCK11 PLPBP NACC2 CNNM2 CSNK1G3 SAV1 GPR180 ING1 SREK1 TBRG1 FLCN ELK4 CDC42SE2 SSU72 NACC1 NFIA DNAJB4 SLC30A7 PEA15 PAQR3 RNF168 CDC25A NAA15 CITED2 RAD21 ARF6 PDZD8 SPRED1 NDEL1 TMEM170A GATAD2A YIF1B PAFAH1B2 IRF2BP2 AXIN2 HIC2 LUZP1 B3GNT2 KIF5B CDCA4 BCL2 PTEN TMCC1 SNCG AGFG1 PHC3 PLEKHF2 JAKMIP2 MEX3C PAWR ERN1 PER1 C16orf72 PRR14L PURA ANKFY1 ARL4C SPATS2L STK40 DAPK1 SLC39A10 AP2A1 CYRIA SOWAHC TLK1 MDM4 FAM229B SYS1 ZBTB10 CFAP45 |

**Table 4.** Differently Expressed and Up-regulated Circulating MEG3 and NEAT1 lncRNAs in MM patients

| Set | Count | LncRNA | Simpson | P-value | FDR | Bonferroni | Jaccard |
|---|---|---|---|---|---|---|---|
| gastric cancer | 31 | CBR3-AS1;DLEU2;DRAIC;DUXAP8;FOXD2-AS1;GAS6-AS1;GIHCG;HAND2-AS1;HCG18;HMGA1P4;HOXA-AS2;HOXC-AS3;INHBA-AS1;KRT7-AS;LINC00565;LINC00662;LINC01006;**MEG3**;MIF-AS1;MIR22HG;**NEAT1**;**PCAT1**;PCAT6;SNHG17;SNHG5;SNHG7;TMPO-AS1;TP53TG1;TPT1-AS1;VPS9D1-AS1;XIST | 0.106 | 1.15E-30 | 6.10E-29 | 6.10E-29 | 0.0543 |
| hepatocellular carcinoma | 30 | ASB16-AS1;DLEU2;DUXAP8;FOXD2-AS1;GAS6-AS1;GATA3-AS1;GIHCG;HAND2-AS1;HOXA-AS2;HOXB-AS3;LEF1-AS1;LINC00174;LINC00205;LINC00662;LINC01134;LINC01139;LINC01224;LINC01551;**MEG3**;MINCR;MIR22HG;MIR503HG;**NEAT1**;NKILA;**PCAT1**;PCAT6;SBF2-AS1;SNHG5;SNHG7;XIST | 0.0971 | 1.54E-28 | 4.08E-27 | 8.16E-27 | 0.0509 |
| gallbladder cancer | 3 | HOXA-AS2;**MEG3**;MINCR | 0.143 | 0.00018 | 0.00024 | 0.00959 | 0.00917 |
| **multiple myeloma** | **3** | **MEG3;NEAT1;PCAT1** | 0.115 | 0.00035 | 0.00045 | 0.0184 | 0.00904 |

## 4. Discussion

In our study, datasets containing MM patient gene expression profiles were used, and we aimed to detect differentially expressed biomarkers such as miRNAs and lncRNAs in circulating tumor cells compared to the ones expressed in bone marrow-derived plasma cells. We focused on upregulated miRNAs and lncRNAs, and we detected there were 4841 upregulated protein-coding genes, 16 upregulated miRNAs and 309 upregulated lncRNAs in CC patients (Table 1; Fig. -1 A, 1B, 1C; Fig. 2A, 2B). Our findings show that liquid biopsy using circulating tumor cells, a non-invasive method, may have advantages in terms of genetic expression patterns compared to bone marrow aspiration and biopsy. In conclusion, it has been shown that the liquid biopsy technique can add newly introduced or verifiable circulating biomarker genes to clinical diagnosis.

Differentially expressed miRNAs in circulating tumor cells compared to bone marrow biopsy samples in MM

Since lncRNAs and their interactions with miRNAs are very important in cancers, our study focused on non-coding genes that were detected as upregulated. In Table 2, differently expressed and upregulated circulating 16 miRNAs in MM patients were shown, and we have determined that only miR-429, miR-210, miR-15a, miR-1180, and miR-103a-2 were related to molecular mechanisms in MM progression such as invasion/migration, hypoxia, cell growth suppression/apoptosis, tumour suppression, and drug resistance respectively. Furthermore, these findings have been indicated only in a few numbers of research papers (17, 18, 19, 36) and in the review article published in 2023, there are no miRNAs that we detected among the miRNAs found to have a role in MM (12).

For example, in a newly published review article in 2020, the role of miR-429 in MM was not mentioned, although it was associated with many cancer types, such as breast cancer, cervical cancer, and prostate cancer (39). In addition, Yong Ming et al. showed in their study published in 2022 that miR-429 plays a role in the Bmi1/AKT pathway that regulates invasion and migration in the MM cell line (17). To the best of our knowledge, there are no clinical studies for miR-429 in MM. Therefore, this miRNA detected in the circulating and differentially upregulated MM can be targeted while planning personalized treatment options and is very important in the treatment stages. Among the circulating miRNAs shown to play a role in different stages of MM, including miR-429, the miRNAs we detected in our study are absent. The only miRNA observed to be common is miR-15a, which has been shown to be downregulated (4, 40, 41). However, in our study, miR-15a was found to be upregulated.

Furthermore, according to the results of our study, the miRNA associated only with circulating upregulated genes in CC patients is Hsa-miR-103a-3p. In our study, hsa-miR-103a-2 was detected as an upregulated circulating miRNA, and circulating protein-coding genes upregulated by targeting hsa-miR-103a miRNA can be suppressed for the efficient treatment in MM. As seen in Table 2, hsa-miR-103a-2 has been associated with drug resistance in MM in the literature, and further clinical studies should be performed. The targets of Hsa-miR-103a-3p were also detected among upregulated genes in Table 3, and these should also be verified in laboratory

conditions.

Differentially expressed MEG3, PCAT1, NEAT1 lncRNAs in circulating tumor cells compared to bone marrow biopsy samples in MM

In our study, the relationship of upregulated 309 circulating lncRNAs with many cancer types is shown in Fig. 3, and only 3 of these 309 lncRNAs are listed at the end of the table obtained as a result of enrichment analyses in which they play a role in multiple myeloma and are not reflected in the graph (Table 4, Fig. 4). According to our results, we showed that these lncRNAs (MEG3, NEAT1, PCAT1) have been shown to be involved in different biological pathways such as prognosis, metastasis, proliferation, migration, apoptosis, and invasion in carcinogenesis in MM patients (Table 5).

**Table 5.** Differently Expressed and Up-regulated Circulating lncRNAs can be related to cancer pathways in MM patients

| Cancer Pathways | Up-regulated Circulating lncRNAs | Simpson | P-value | FDR | Bonferroni | Jaccard |
|---|---|---|---|---|---|---|
| prognosis | FGF14-AS2, FOXD2-AS1, GAS6-AS1, HOXA-AS2, LINC00574, LINC01002, **MEG3**, NEAT1, NKILA, **PCAT1**, PCAT6, SNHG10, TMPO-AS1, XIST | 0.0864 | 2.71E-13 | 1.90E-12 | 1.90E-12 | 0.0306 |
| metastasis | KRT7AS, LINC00963, **MEG3**, MINCR, **NEAT1**, NKILA, PCAT6, SBF2-AS1, XIST | 0.209 | 1.38E-12 | 4.83E-12 | 9.66E-12 | 0.0262 |
| proliferation | DLEU2, HOXAAS2, KRT7AS, LINC00963, **MEG3**, MINCR, **NEAT1**, **PCAT1**, PCAT6, PICSAR, SBF2-AS1, SNHG5, XIST | 0.0663 | 5.54E-11 | 1.29E-10 | 3.88E-10 | 0.0264 |
| migration | LINC00963, **MEG3**, MIR503HG, **NEAT1**, **PCAT1**, PICSAR, SBF2-AS1, XIST | 0.063 | 4.33E-07 | 7.58E-07 | 3.03E-06 | 0.0187 |
| apoptosis | HOXAAS2, LINC00963, **MEG3**, **NEAT1**, **PCAT1**, PCAT6, XIST | 0.0745 | 7.44E-07 | 1.04E-06 | 5.21E-06 | 0.0177 |
| invasion | **MEG3**, MIR503HG, **NEAT1**, **PCAT1**, SNHG5, XIST | 0.0517 | 3.78E-05 | 4.41E-05 | 0.00027 | 0.0143 |

There are 354 research articles on lncRNA MEG3 and its role in various diseases, including cancers, on the "genecards.org" website, an official website where information about the genetic location of genes and the diseases related to genes and their products are shared. Among these 354 articles, it is seen that there are only three articles that mention the role of lncRNA MEG3 in MM (https://www.genecards.org/cgi-bin/carddisp.pl?gene=MEG3; on 13.02.2023). MM is a type of cancer known to have impairment in osteogenic differentiation of mesenchymal stromal cells (MSCs), and Wenzhuo Zhuang et al. showed that the lncRNA MEG3, which was found to be downregulated in MM, played a role in the activation of BMP4 transcription in bone marrow samples (42). In another study, lncRNA MEG3 was shown to increase bortezomib sensitivity by inhibiting autophagic pathways in MM cell lines (43). According to our knowledge, in the only published study on the association of LncRNA MEG3 with miRNAs in MM, it is proposed that downregulated lncRNA MEG3 may inhibit the progression of tumorigenesis by interacting with overexpressed miR-181a (44). Contrary to these studies, our study found that lncRNA MEG3 expression levels were upregulated in circulating tumour cells in MM, and it was shown that it is also important in all pathways related to cancerization in MM.

Studies by Yin Gao et al. have shown that lncRNA NEAT1 reduced miRNA-214 gene expression by overexpressing B7-H3, a checkpoint molecule that inhibited immune responses that develop specifically to tumor antigens in MM. Thus, by suppressing the JAK2/STAT3 signaling pathway, it has been observed that M2-polarized macrophages accelerate cancer in MM cell lines and clinical specimens (12, 45). In addition, it has been reported that the interaction of lncRNA NEAT1 and miRNA-193a/MCL-1 plays a role in MM cell lines, dexamethasone resistance, which is one of the first anticancer agents used in the treatment of MM and is associated with poor prognosis (12, 46). Another study showed that lncRNA NEAT1 may interact with miR-125a, which has been downregulated in MM, and act as a new biomarker for determining treatment response, survival profiles and stage of disease in MM. The aforementioned study was conducted with clinical samples, and it was found that NEAT1 gene expression levels increased in individuals diagnosed with MM compared to healthy individuals, and this situation was closely related to survival times (47). Compared with other haematological cancer types, Elisa et al. showed that lncRNA NEAT1 expression levels were higher in MM cell lines, mouse experiments, and MM patient samples and found that NEAT1 had a role in the DNA repair mechanism (48). Also, on the "genecards.org" website, lncRNA NEAT1 has been associated with non-cancer diseases and the type of cancer it is most associated with is gastric adenocarcinoma (https://www.genecards.org/cgi-bin/carddisp.pl?gene=NEAT1; on 13.02.2023). On the website where the research articles on the NEAT1 gene are listed, only four scientific articles mentioned above explaining the role of NEAT1 in MM, among a total of 439 articles, are mentioned.

**Table 6.** Drugs and drug components targeting MEG3, NEAT1 and PCAT1 lncRNAs

| Set | Count | LncRNA | Simpson | P-value | FDR | Bonferroni | Jaccard |
|---|---|---|---|---|---|---|---|
| Panobinostat | 64 | NEAT1, PCAT1 | 0.207 | 2.16E-14 | 2.60E-13 | 5.18E-13 | 0.0143 |
| Topotecan | 60 | NEAT1, PCAT1 | 0.194 | 2.17E-14 | 2.60E-13 | 5.21E-13 | 0.0148 |
| L-685458 | 54 | PCAT1 | 0.175 | 1.68E-13 | 1.34E-12 | 4.03E-12 | 0.0151 |
| PD-0332991 | 52 | PCAT1 | 0.168 | 9.75E-12 | 5.85E-11 | 2.34E-10 | 0.014 |
| PF2341066 | 46 | PCAT1 | 0.149 | 1.68E-10 | 8.06E-10 | 4.03E-09 | 0.0139 |
| Sorafenib | 42 | PCAT1 | 0.136 | 3.94E-10 | 1.58E-09 | 9.46E-09 | 0.0142 |
| Irinotecan | 46 | NEAT1, PCAT1 | 0.149 | 5.23E-10 | 1.79E-09 | 1.26E-08 | 0.0135 |
| Nutlin-3 | 29 | PCAT1 | 0.0939 | 7.04E-10 | 2.11E-09 | 1.69E-08 | 0.0171 |
| TKI258 | 33 | NEAT1, PCAT1 | 0.107 | 1.23E-07 | 2.95E-07 | 2.95E-06 | 0.0132 |
| Paclitaxel | 33 | PCAT1 | 0.107 | 2.11E-07 | 4.60E-07 | 5.06E-06 | 0.0129 |
| Lapatinib | 32 | MEG3, PCAT1 | 0.104 | 3.02E-07 | 6.04E-07 | 7.25E-06 | 0.0129 |
| AZD0530 | 27 | MEG3 | 0.0874 | 8.30E-06 | 1.42E-05 | 0.000199 | 0.0119 |
| AZD6244 | 27 | PCAT1 | 0.0874 | 1.14E-05 | 1.82E-05 | 0.000274 | 0.0118 |
| PD-0325901 | 30 | PCAT1 | 0.0971 | 3.58E-05 | 5.05E-05 | 0.000859 | 0.0107 |
| 17-AAG | 22 | NEAT1, PCAT1 | 0.0712 | 4.15E-05 | 5.53E-05 | 0.000996 | 0.0118 |
| Erlotinib | 19 | NEAT1 | 0.0615 | 0.00044 | 0.000528 | 0.0106 | 0.0107 |
| cisplatin | 2 | MEG3 | 0.111 | 0.00403 | 0.00806 | 0.00806 | 0.00615 |

Another lncRNA found to be upregulated in circulating tumour cells in MM patients in our study was PCAT1. While there are articles about the relationship of lncRNA PCAT1 with cholangiocarcinoma, bladder cancer and squamous cell carcinoma on the "genecards.org" website, only 1 article is mentioned showing the role of lncRNA PCAT1 in multiple myeloma (https://www.genecards.org/cgi-bin/carddisp.pl?gene=PCAT1; on 13.02.2023). A study conducted with MM patient samples showed that serum lncRNA PCAT1 expression levels were higher than the serum values of healthy individuals (49). The expression levels of lncRNA PCAT1 were higher in BM aspirate samples of a patient diagnosed with asymptomatic MM compared to healthy individuals (50). To our knowledge, there is no article on the interaction of lncRNA PCAT1 with any miRNA and the association of this interaction with MM.

Drugs specifically targeting MEG3, PCAT1, and NEAT1 lncRNAs in only circulating tumor cells, not in bone marrow biopsy cells of MM patients Drugs and drug components found to target MEG3, PCAT1 and NEAT1 lncRNAs in Table 6 were determined in our study, and these drugs can be tested in clinical studies, and the expression of upregulated lncRNAs can be suppressed. Only 2 of the drugs shown to interact with MEG3, PCAT1, and NEAT1 lncRNAs in our study, Palbociclib (brand name Ibrance®, original name PD-0332991), Panobinostat (brand name Farydak®, original name LBH589) are among the FDA-approved medications on the International Myeloma Foundation website. (https://www.myeloma.org/multiple-myeloma-drugs). Also, Panobinostat and Palbociclib are unavailable on the National Cancer Institute website (https://www.cancer.gov/aboutcancer/treatment/drugs/multiple-myeloma). In addition, the fact that Topotecan, L-685458, PF2341066, Sorafenib, Irinotecan, Nutlin-3, TKI258, Paclitaxel, Lapatinib, AZD0530, AZD6244, PD-0325901, 17-AAG, Erlotinib, Cisplatin drugs are not in this list highlights the originality of our study. These drugs need to be confirmed by clinical data.

In summary, considering the findings in our study, despite all these limited studies, it is possible to use lncRNA MEG3, NEAT1, and PCAT1 as a biomarker in the diagnosis of MM and as a target gene in the treatment steps of MM. In addition, there seems to be a limited number of studies on the interactions of lncRNAs with miRNAs, including those involved in MM. Studies should be diversified and supported by clinical and wet lab studies, and interactions and related biological pathways should be determined.

Finally, our study has some limitations. For example, we only have data on newly diagnosed MM patients, and bioinformatic analyses should be performed for patients diagnosed with other stages of MM and under different treatments. In addition, the number of studies on the miRNAs and lncRNAs is very limited, and some studies share their findings on cell lines, not clinical findings. The data obtained by bioinformatic analyses should be verified in clinical samples, and a direct comparison of bone marrow biopsy and liquid biopsy should be made.

According to our results, different upregulated lncRNAs and miRNAs not previously associated with multiple myeloma were detected in the circulating tumor cell. The fact that miRNAs and lncRNAs, which have been shown to play a role in MM, have also been detected in tumor circulating cells, indicating that technically easier liquid biopsy may replace BM aspirate and biopsy. The presence of miRNAs and lncRNAs that have not yet been shown to be related to MM in our study constitutes the novel part of our article, and our study is unique in this respect.

**Conflict of interest**
Authors declare that there is no conflict of interest.

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None to declare.

**Authors' contributions**
Concept: G.A.D., Design: G.A.D., Data Collection or Processing: G.A.D., Analysis or Interpretation: G.A.D., Literature Search: G.A.D., Writing: G.A.D.

**Ethical Statement**

Publicly available datasets of 1046 MM patients and 22 healthy controls were used. Therefore, ethics committee approval is not required. Publicly available datasets were analysed analyzed in this study. This data can be found here for GSE164701 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE164701), GSE24080 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24080), GSE2658 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE2658), and GSE5900 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5900).

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