Original Article

Detection of New Single Nucleotide Polymorphisms in Plasma Cells Carcinoma (Myeloma) by Polymerase Chain Reaction–Single Strand Conformation Polymorphism and Sequencing Technical Methods

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Abstract

Background: MicroRNA (miRNA) is a short, single-stranded, non-coding sequence of RNA that does not translate into proteins. Because of their ability to control gene expression, recent studies have shown that these short, non-coding sequences play an important role in cancer. So that, detection the polymorphisms or variants in miRNA genes may help to identify their correlation to cancer susceptibility. **Objectives:** Study the polymorphisms in miRNA genes or variants miRNA genes and their correlation with myeloma disease. **Materials and Methods:** Twenty samples were collected from people who visited Marjan Hospital in Province of Babylon, Iraq, from February to July 2022. In addition, 20 samples from healthy people were collected as control. Genotyping of miRNA was accomplished using the polymorphism techniques of polymerase chain reaction and single strand conformation polymorphism (SSCP). DNA sequencing was also applied to categorize all the ranges of SSCP models detected using only gel imaging. **Results:** The genotypes obtained in this experiment verified that several single nucleotide polymorphisms were obtained between 5-band and 6-band miRNA National center for biotechnology information Primer3 plus reference. **Conclusion:** The results explain that the miRNA gene polymorphisms may have correlation with the susceptibility to myeloma disease.

Keywords: Multiple myeloma, sequencing, PCR–SSCP, polymorphisms

Introduction

Multiple myeloma ranks the second most prevalent illness of blood's malignancy.[1] This disorder has a property of accumulated-plasma blood cells inside bone marrow (BM) with the existence of a lone clone of multiple myeloma immunoglobulin A in serum or urine.[2] Multiple myeloma is typically accompanied by an inconspicuous condition called pre-malignant monoclonal gammopathy of undetermined significance (MGUS), generally diagnosed with clonal BM plasma cells infiltration < 10% and serum protein M < 30 g/dL, with no signs of anemia, impaired function of the kidney, excessive calcium in the blood, or bone lytic lesions and amyloidosis.[3,4]

The causes behind such a disease are typically genetic variations, or the presence or lack of chromosomes in addition to point mutations.[5] Report cases of Multiple myeloma revealed cytogenetic changes such as deletion of p53 (deletion of 17p) or genetic abnormalities that may lead to abnormal cell signaling pathways. The main mutable genes in multiple myeloma cases are TP53 (8%), DIS3 (11%), FAM46C (11%), NRAS (20%), KRAS (23%); TRAF3, RB1, BRAF, CYLD, ACTG1IRF4, PRDM, HIST1H1E, MAX and EGR1,[6] which are variable but

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less frequency effective. It has been supported by other research works related to DNA methylation, chromatin's structure and loss of miRNA regulation, which could play a role in the progression of multiple myeloma.[3]

miRNAs are a type of small untranslated nucleotide in the range of 20–25 nucleotide. The mature miRNAs are typically associated with their targets in three untranslated areas, and gene expression regulation of the goal gene is regulated by translation halting or mRNA degeneration. Moreover, miRNAs play an important role in controlling the genetic expression needed to control normal functions of cells. The abnormal expression in miRNAs was observed in human malignant diseases and phenotypic changes including multiple myeloma.[7]

An abnormal expression in miRNAs can cause tumor formation by disrupting the mechanism that regulates the cell's progra. An abnormal expression in miRNAs can cause tumor formation by disrupting the mechanism that regulates the cell's programed death; aberrant expression of miRNAs has been entangled with several human diseases and phenotypic dissimilarities like multiple myeloma.[7,8]

This study was aimed to identify the polymorphisms in miRNA genes or variants miRNA genes and their correlation with myeloma disease.

Materials and Methods

Sampling

A total of 20 blood samples had been gathered from patients with multiple myeloma who went to most cancers center, Babylon Province, Iraq and 20 samples as control.

DNA extraction

DNA from whole blood cells was extracted and purified by using DNA Extraction and Purification Kit (FAVORGEN, Taiwan).

Polymerase chain reaction–single strand conformation polymorphism amplification

Specific primers were used to amplify targeted DNA sites (the primers were used to recognize miRNA) were: The forward primer: 5′ CCCCTTCCCTTCTCCTCCA 3′, reverse primer: 5′ CGAAAACCGACTGATGTAAC 3′.

The PCR reaction was 20 μL reaction included: 1.5 μL of forward- and reverse-primer, 3 (μL) template-DNA, 12.5 (μL) of master-mix (Bioneer), and 5 μL of nuclease-free water. Amplification program was: the initial denaturation 94°C for 5 min; 94°C into 30 cycles for (1 min), annealing temp. at 58.7°C for 1 min and extension temp. 72°C for 1 min; final extension was 72°C for 5 min. Amplification products were run on agarose gels, stained by ethidium bromide, and visualized by UV light.

The PCR-SSCP technique includes the following steps: PCR gene fragment amplification, non-denaturing page resolution and photography illustration of ethidium bromide use. The PCR amplification method is similar for all SSCP primers used, except for the annealing temperature, which differs according to the primers.[9]

Statistical analysis

Data analysis was accomplished by SPSS software program system (version 17.0, SPSS Inc., Chicago, Illinois)) where P-values < 0.05 was considered to be statistically significant.

Ethical approval

The study protocol, the subject information, and patients' consent forms were reviewed and approved by a local ethics committee according to the document number 54 at November 19, 2021 to get this approval.

Results

Genotyping study

Figure 1 explains the amplification product of miRNA gene and Figure 2 shows results of SSCP-PCR miRNA 149bp amplified product polyacrylamide gel type-electrophoresis.

Selected lanes 1–15 refer to micro RNA's PCR-SSCP pattern (SSCP analysis involves the following four steps: (1) polymerase chain reaction (PCR) amplification of DNA sequence of interest; (2) denaturation of double-stranded PCR products; (3) cooling of the denatured DNA (single-stranded) to maximize self-annealing; (4) detection of mobility difference of the single-stranded). "symbol C" indicates to the control but "symbol P" indicates to the patients' groups. All the gained SSCP

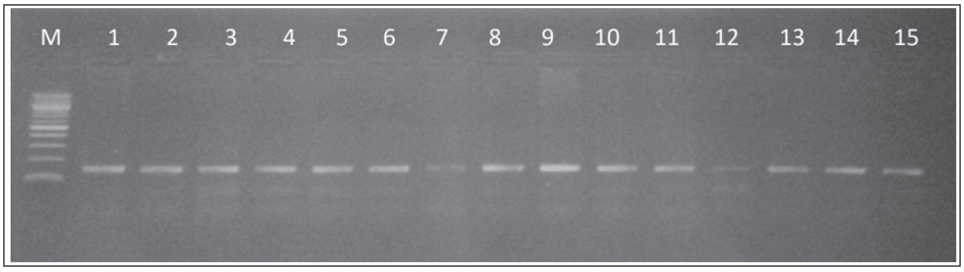

Figure 1: Agarose-gel electrophoresis of amplified products of microRNA gene for normal and patients (from 1 to 11 no. sample for patient and from 12 to 15 no. sample for control) M; refers to DNA ladder, lanes 1–15 refer to the patterns of amplified products of micro RNA (149 bp)

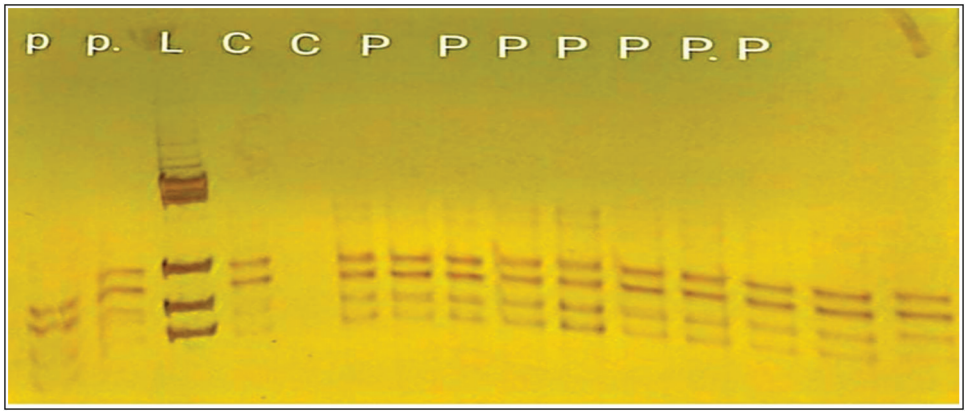

Figure 2: DNA polymorphisms of microRNA gene of myeloma patients by electrophoresis pattern of PCR-SSCP, 20 A, 100 V and 15% gel

Table 1: Distribution of DNA polymorphism of the miRNA gene by different-number of bands, their association with myeloma patients, and groups of control

DNA polymorphism of MIRNA196A2 gene	Group of patients No. (%)	Group of control No. (%)	*P*-value	OR	95% CI
5 bands	15 (75%)	19 (95%)	0.021*	0.44	0.30–0.64
6 bands	5 (25%)	1 (5%)			
Total number	20	20			

*$P \leq 0.05$

gels were matched with each other to show how many haplotypes were identified in SSCP gels where SSCP band pattern styles were found. The single stranded DNA bands (ssDNA) were found to take up the top section of the agarose-gel (polyacrylamide are smaller than those of agarose. This makes it more suitable for the separation of proteins or RNA fragments). As a result, double stranded-DNA took up the down section of the agarose-gel. The ssDNA varieties in SSCP gels rely on the discovery of both amplified genetic pattern and the environment of single strand conformation polymorphism (PCR-SSCP).

Following the amplification of the target site, the miRNA gene's conformational polymorphism using PCR-SSCP technique. All the SSCP gels collected were aligned to disclose the number of haplotypes obtained. The implications have been observed for the inclusion of the two haplotypes of SSCP band patterns in SSCP gels. The version of ssDNA in SSCP gels should be aware of each amplicon's genetic sample.

But it may be complex to establish whether the sample of all solved SSCP bands utilize only the vision of the gel. Those DNA polymorphisms can, therefore, be demonstrated through the use of a sequencing technique [Table 1]. The result of sequencing showed the several SNPs between the one haplotype resolved by using the primer miRNA in addition sequences of reference. The results of Figure 3 showed the presence of two SNPs. Which revealed that which located at position 50 (C/T), position 51 (T\A), position 52 (C\A), position 62 (A\T), position 63 (T\C), position 64 (C\G), position 125 (C\T), and position 127 (T\G) as a substitution mutation according to the reference sequence alignment of the human miRNA gene [Figures 4 and 5].

Discussion

The results of the miRNA-gene polymorphism showed that two different haplotypes including 5 plus 6 bands, also the results of sequencing showed that a number of (SNPs) were observed through each band. Contrariwise, the SNPs were detected in patients groups of myeloma patients and controls between two groups. Results indicate that 5–6 bands were associated with myeloma compared to the control group.

It has been shown that miRNAs play a major role in multiple myeloma evolution by Al Masri *et al*.,[10] who were able to prove that cell lines of multiple myeloma disease and samples of multiple myeloma patients showed a gene expression less than of miRNA-125b, miRNA-133a, miRNA-1, miRNA-124a, miRNA-15, miRNA-196a2 and miRNA-16 than their ordinary counterparts. It has been shown that the distinct gene expression of many miRNAs between monoclonal unlimited significance multiple myeloma gammopathy (MGUS) and multiple myeloma plasma-cells as well as ordinary plasma-cells has also been proposed as a function for miRNAs in multiple myeloma advancement.[11]

Lionetti *et al*.[12] were able to analyze gene transcription and DNA copy amount and miRNAs in cells of multiple myeloma patients and plasma cells collected from multiple myeloma cases making use of unified high-resolution microarray analysis. They were capable of discovering 16 different varieties of miRNAs (miR-22 at 17p13.3, miR-106b, miR196a2 and miR-25 at 7q22.1, miR-15a at 13q14.3, miR-21 at 17q23, and miR-92b at 1q22) and other types positioned in different regions of the chromosomes from mechanisms that are usually affected by deletion or transmission of chromosomes in multiple myeloma

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          10        20        30        40        50        60        70        80        90       100
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
refrenceCCCCTTCCCTTCTCCTCCAGATAGATGCAAAGCTGAATCTCCCG
CCCTGCTCGCTCAGCTGATCTGTGGCTTAGGTAGTTTCATGTTGTTGGG
ATTGAGT
sample1 .....................................................T...........T.G.....................................
sample2 .....................................................TAA.........T.G.....................................
sample3 .....................................................TAA.........TCG....................................

          110       120       130       140
          ....|....|....|....|....|....|....|....|....|....
refrenceTTTGAACTCGGCAACAAGAAACTGCCTGAGTTACATCAGTCGGT
TTTCG
sample1 ........................T.G.....................
sample2 ........................G.....................
sample3 ........................G.....................
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Figure 3: Sequences alignment ID: AF490843.1 results for *Homo sapiens* 3p mature miRNA region of miRNA196A2 gene fragment by Bio Edit program version 7.2.5

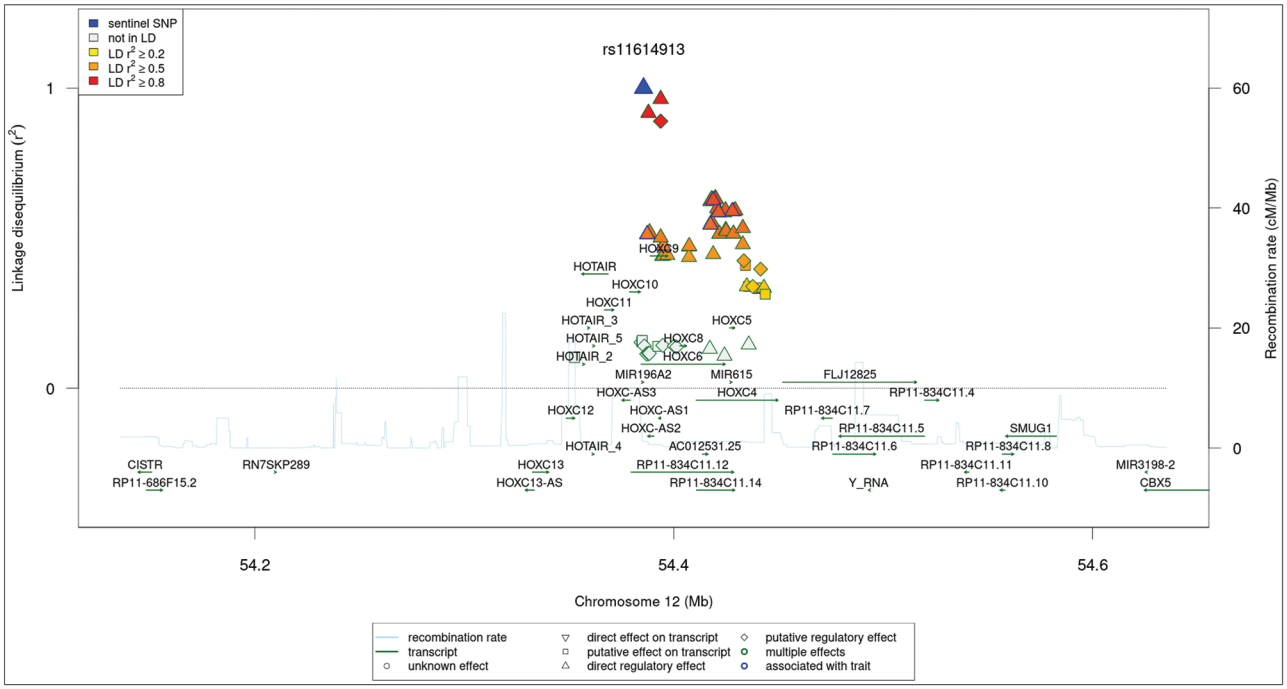

Figure 4: Linkage of loss of equilibrium plots shows the quantity of correlation between a lookout variant and its vicinity variants. The *y*-axis displays correlation coefficient (r^2), the *x*-axis exhibits chromosomal position of every SNP

patients where an increase or decrease in the expression of plasma cells in multiple myeloma patients was observed in comparison with ordinary plasma cells.[12] The relation of gene expression increment of miRNA positioned at 1q, such as miR-1231, miR-205, miR-215, and miR-488, was related with chromosome 1q gain, that is recognized in more than 50% of patients.[12]

Gutierrez *et al*.[13] pointed out the loss of regulation of the miRNA gene transcription in dissimilar genetic subtypes of multiple myeloma patients where they found a genetic expression much higher than miR-.1 and miR-.133 a in multiple myeloma with t (14; 16), that is named high risky cytogenetic., and the down organizing miR-.135b and miR-.146a in multiple myeloma with t. (4; 14), as

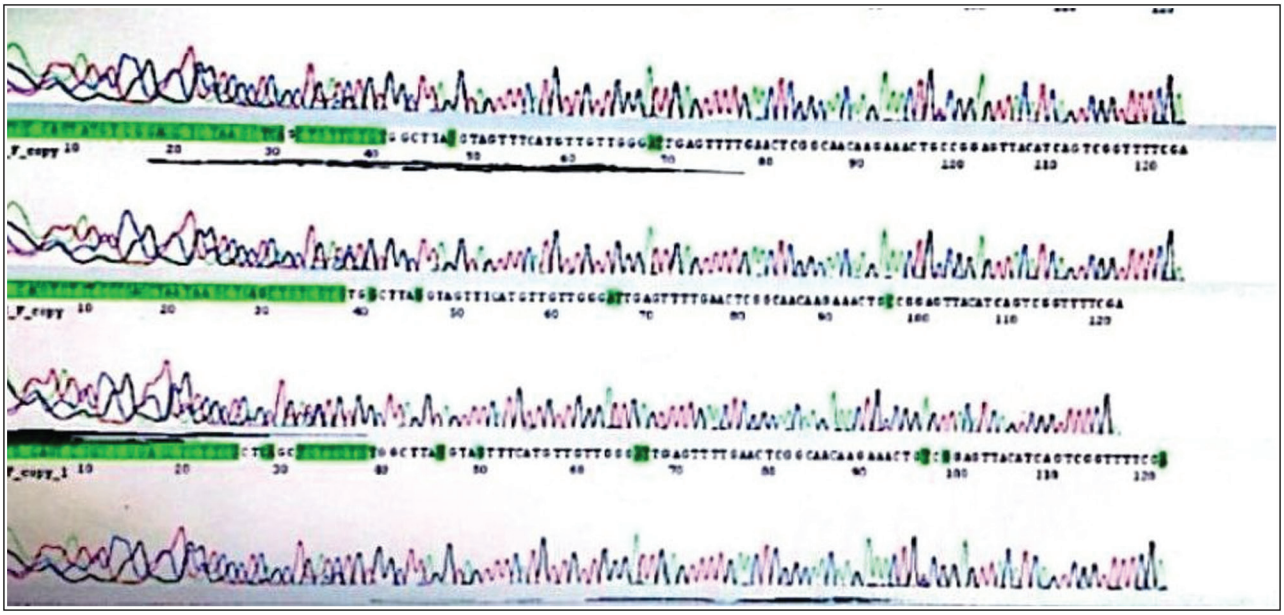

Figure 5: Chromatogram data for samples alignments

well as goal genes participating in the IL-.1 signaling pathway.

Chi *et al*.[14] recognized exact miRNA inscriptions connected with the more familiar 1gH translocations t (4; 14) and t (11; 14) and deletion (del) (13q).[14]

Conclusion

These obtained results explain that the changes of miRNA gene polymorphisms have correlation with the susceptibility to myeloma disease.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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