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Genomic Landscape of a Case of Multiple Myeloma by Whole Exome Sequencing

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ABSTRACT

Multiple Myeloma (MM) is the malignancy of plasma cells that results in organ damage**.** These subjects diagnosed with MM are in need of new treatment strategies to improve better outcome. Whole exome sequencing (WES) was performed on the blood sample of a 47 years old male subject, diagnosed with MM in 2010. Analysis of WES data to identifying driver and passenger mutations was performed using various tools and algorithms. Analysis revealed that translocations in *IGH* and *IGK* loci found to be primary aberrations in this case. Prioritizing the screening for those genes that are known to be secondary mutations led to identification of critical tumor suppressor genes, *KMT2C (MLL3*), *BCL2, NOTCH2* and *DIS3* as driver mutations. We also identified list of mutations that indicted secondary abrasions are continually happen during clonal expansion in MM. Myeloma is individual specific and each patient is unique. Therefore, the genomic data of each case and relevant clinical feature would be important guidelines for treatment strategies.

KEYWORDS: Multiple Myeloma, WES, Translocation, Driver and Passenger Mutations, Treatment

INTRODUCTION

Myeloma is a cancer formed by the malignant proliferation of monoclonal plasma cells in the bone marrow producing a distinct tumor called plasmacytoma. Presence of multiple such plasmacytomas across bones is regarded distinctively as MM (Dimopoulos *et al*, 2014). Clinical manifestation of MM includes anemia, hypercalcaemia, renal dysfunction, monoclonal gammopathy and light chain amyloidosis (Gerkes *et al*, 2007). Genetic anomalies of MM can be of two types based on the chromosomal aberrations. Hyperdiploidy in MM is observed on those malignancies that involves trisomies of odd numbered chromosomes, 3, 5, 7, 9, 11, 15, 19, and 21, while non-hyperdiploidy is distinguished by the presence of inter-chromosomal translocations involving 14q32 regions bearing immunoglobulin heavy chain alleles with other partner chromosomes bearing enhancer or active chromatin regions (Rajan *et al*, 2015).

MM is a genetically heterogenous disease manifested through a multistep process of plasma cells accumulating genetic "hits" over time, initiating malignancy (Morgan *et al*, 2012). In this multistep process, inherited driver gene mutations begin by sensitizing the somatic cells resulting in acquiring additional somatic changes, that are either hyperdiploid or nonhyperdiploid changes representing the early initiating mutagenic events leading to plasma cell immortalization and disease initiation (Pon *et al*, 2015). These multiple molecularly-defined subtypes subsequently accumulate more secondary aberrations including duplications, deletions, additional translocations, mutations, and epigenetic modifications. The gradual accumulation of such genetic "hits" in genes of tumor suppressor and oncogenic pathways results in the deregulation of fundamental processes of plasma cells damaging the genomic integrity leading to plasma cell immortalization, driving malignancy (Colombo *et al*, 2013). Accumulation of such genetic aberrations occurs at varying rates in diverse subclonal populations and brings in intra tumor genetic heterogeneity followed by recurrent mutations occurring either early or late in the evolution of tumors (Lohr *et al*, 2014).

Studies have identified several hundreds of genes as key drivers for initiating and manifesting MM. These genes belong to Ras/MAPK, phosphatidyl/inositol 3-kinase/Akt (PI3K/Akt), notch, wingless (WNT), and nuclear factor-kappa B (NF-κB) pathways and mutations in genes of these pathways contribute towards sustained activation in MM cells (Bommert *et al*, 2006). At each step of the transitioning phase in this multistep process and acquisition of novel mutations is always followed by subclonal evolution from reservoir clones with branching patterns. Each of these subclones evolve by accumulating common and novel mutations beginning the asymptomatic phase of monoclonal gammopathy of undetermined significance (MGUS), progressing linearly towards the clinical course and drug sensitivity resulting in terminal phases such as extramedullary tumors, relapse and/or leukemic conversion (Furukawa *et al*, 2015).

The first set of interactions between the genetic defects of B-cells (Pro B-Cell) with bone marrow microenvironment (BMM) followed by interactions between the genetic defects of mature B-cells with germinal centers of the secondary lymphoid organs are the crucial factors governing the pathophysiology and malignant growth of multiple myeloma (MM). These interactions lead to the activation of MM signaling pathways promoting the expansion of the malignant clones, stimulating neoangiogenesis and osteoclastogenesis (Bolli *et al.*, 2018).

In the current study, we performed whole exome sequencing (WES) investigations in a severe case of MM and identified driver gene mutations. These mutations were found to precede, succeed or jointly initiate several stages of activations in oncogenic pathways contributing towards sustained activation in MM cells.

Ethical approval and consent to participate

Written informed consent was obtained from MM case. The study protocol received ethical approval from the Ethics Committee of the University of Mysore (IHEC-UOM No. 147/Ph.D/016/17).

MATERIAL AND METHODS

A male case, aged 47 years was diagnosed with MM in 2010 by clinicians and confirmed with oncologist. A total of 5 ml EDTA blood was collected from a case and genomic DNA was extracted using a Promega Wizard® Genomic DNA purification kit. The isolated DNA was quantified by Bio-photometer and gel electrophoresis.

Whole Exome Sequencing and Alignment

Whole exome sequencing was done at coverage of 100x by using illumine HiSeq 2500 system. The FASTQ file was aligned against hg19 build of the human reference genome on Strand-NGS. For aligning the exome sequences, Strand-NGS was used in the current study due to its accuracy in terms of percent correctly mapped reads and receiver operating curves. The reds were aligned against the whole exome build hg19 using Strand NGS v3.3.1 (Strand Life Sciences, Bengaluru, India).

Post alignment quality check was performed to remove bad variants. Further, variants were called using the strand-NGS variant calling program and the VCF files with Single Base Variants (SBVs) and Multi Base Variants (MBVs) were exported. The VCF files containing SBV and MBV were annotated based on position, gene and amino acid change, zygosity and mutation effects. We collated the data from all the four annotators and the variant calls agreeing with all of the annotator programs along with those variants having higher read depth (>30) were included in the study.

For structural variation analysis we used tool called DELLY, which integrates all types of genomic rearrangements such as, short insert paired-ends, long-range mate-pairs and split-read alignments. For detecting copy-number variable deletion and tandem duplication DELLY is powerful tool. It also enables to detect balanced rearrangements such as inversions or reciprocal translocations. DELLY compare NGS real data based on 1000 Genomes Project and cancer genomes to reliably uncover SVs, and validation experiments of randomly selected deletion loci showed a high specificity and cost effective (Rausch et al 2012).

Identifying Driver Gene Mutations

Driver gene mutations were identified by subjecting variants into Cancer-Related Analysis of VAriants Toolkit (CRAVAT), a program that currently employs two analysis tools, CHASM and VEST. This predicts the functional significance of mutations observed in the genomes of cancer cells (Douville et al., 2013; Masica et al., 2017). CHASM-3.1 is the most recent version of "Cancer-specific High-throughput Annotation of Somatic Mutations" a method that predicts the functional significance of somatic missense mutations observed in the genomes of cancer cells, allowing mutations to be prioritized in subsequent functional studies, based on the probability that they give the cells a selective survival advantage (Wong et al 2011). We also used recent version of the Variant Effect Scoring Tool (VEST-4). This tool is machine learning method that predicts pathogenic probability of non-silent variants and functional significance of variants (Douville et al., 2016).

RESULTS

The subject MRI scans showed significant spinal cord compression and spinal canal compromise at L1-L2 and L5-S1. He faced difficulties in lying down and in walking. The case also showed increased production of monoclonal immunoglobulins leading to a change in the kappa:lambda light chain quotient. His PET CT scan showed multiple osteolytic metastases in the left ilium, ischium, sternum, sacrum, thoracolumber spine, bilateral ribs, right glenoid, coracoids and in left proximal humerus. MRI of the pelvis showed abnormal signal changes involving L1 and L5 vertebral bodies. MM was confirmed through ultra sound guided core biopsy in the right scapula which showed a round cell tumor. The subject received three cycles of radiation treatment and four cycles of chemotherapy. Bortosomib a proteosom inhibitor and MM panel drugs as common drug for MM cases practiced for him. The subject has a poor disease prognosis and none of the treatments showed any improvements.

WES was done at coverage of 100x and obtained total number of reads (R1+ R2) of more than 26 million. The mean read quality (Phred score) was found to be 27.5 for R1 and 29.03 for R2 with a total of 26630811 reads in both read orientation. The mean read length was 100 bp and a total of 8.3 GB was obtained from sequencing reaction. Table 1 revealed the DNA variations found in a case of MM under study. Annotating the variants from WES revealed a total of 117093 variants consisting of 73.05% of SBVs, 5.9% of MBV/In-Dels, 0.17% of translocations, 0.057% of inversions and 20.8% of large structural variations (Table1).

Table 1: DNA variations based on number and types in a case of MM

S. No.	Type of Variation	Number of Variations	Percentage %
1	Single Base Mutations	37917	73.05%
2	Multi-Base Mutations	3065	5.90%
3	Chromosomal Translocations	92	0.17%
4	Chromosomal Inversions	30	0.057%
5	Structural Variation - Duplications	9988	19.24%
6	Structural Variation- Deletions	810	1.56%

Mutations and polymorphisms were found across exons of several tumor suppressor genes and oncogenes. Annotating these variants revealed several deleterious and damaging driver gene mutations in genes *MLL3* and *LTB* contributing towards early initiating mutagenic events leading to plasma cell immortalization and disease initiation. Focusing on the molecular events driving and sustaining the cancer identified three crucial genomic alterations in genes *RAG1*, *IGH* and *IGK* loci, while a spectrum of genes were identified with several damaging mutations due to secondary aberrations. Mutations in molecularly-defined subtypes included numerous duplications, deletions and additional translocations.

Driver gene mutations

Table 2 gives the identified driver mutations in a case of MM under study.*MLL3* (*KMT2C*) had two heterozygous mutations at read depth (RD) of 130. A mutation, a single base nonsynonymous substitution mutation was found 71kb downstream from the previous mutation, a substitution of base C to T at, 151873293bp (c.C9245T) in exon 38 replacing proline with leucine at 3082nd amino acid, position (p.P3082L) with total read of 72.Another mutation from G to A in chromosome 7, in position 151945007bp (c.G2512A) in exon 14 replacing glycine with serine at 838th amino acid position (p.G838S). These MLL3 mutations were predicted to be deleterious and damaging by both CHASM/VEST programs. LTB was found carrying a missense mutation, C to T (c.C365T) in chromosome 6 at 31548856bp located on exon 4 replacing alanine with valine at 122nd position (p.A122V) (Table 2).BCL2 located in chromosome 18 was found carrying one heterozygous damaging mutation in position 60985773 with rs1800477 and nucleotide change of C to T with alternate reads of 28 and A43T amino acid change and classified as oncogenes (Table 2).

Table 2: List of damaging and heterozygous missense driver mutations in a case of MM

Genes	Chromosome No. & position (bp)	dbSNP	DNA change	Amino acid change
MLL3 (KMT2C)	Chr7:151873293	rs61730545	c.C9245T	p.P3082L
	Chr7:151945007	rs137949498	c.G2512A	p.G838S
LTB	Chr6:31548856	rs2229699	c.C365T	p.A122V
BCL2	chr18:60985773	rs1800477	c.G127A	p.A43T
NOTCH2	chr1:120572547	rs61788900	c.A137G	p.N46S
	chr1:120572572	rs61788901	c.G112A	p.E38K
	chr1: 120611964	rs11810554	c.C57G	p.C19W

NOTCH2 located in first chromosome, was found to carrying three damaging mutations that classified as tumor suppressor genes. First mutation with rs61788900 in position 120572547 with alternate reads of 28 was found heterozygote change of T to C and N46S amino acid change. Second heterozygote mutation was found in position 120572572 with alternate reads of 26 and change of C to

T with rs61788901 and E38K amino acid change. Third heterozygote mutation in NOTCH2 was found in position 120611964 with rs11810554 and alternate reads of 5, G to C nucleotide change and C19W amino acid change (Table 2).

Malignancy translocations

Table 3 provides the damaging translocations observed in a case of MM under study. We identified three genes *RAG1*, *IGH* and *IGK* loci containing non-hyperdiploidy variations. *RAG1* located in chromosome 11 showed a contiguous heterozygous duplication of 221bp in exon 2 from 3536594776bp to 36594997bp truncating the coding structure. Further, immunoglobulin heavy variable locus bearing genes *IGH*, *IGHV3-13*, and *IGHV3OR 16-10* showed inverted non-reciprocal homozygous intra-chromosomal translocation of 444bp size between chromosome t(14;16), from chr14:106586315-106586759 to chr16: 33006280-33006543. Additionally, immunoglobulin kappa locus bearing genes *LOC101929708*, *IGKV1D-43* and *IGK* showed inverted non-reciprocal heterozygous intra-chromosomal translocation of 1.9kb between chromosome t(22;2), from chr22:17385090-17365365 to chr2: 90249300-90249473 (Table 3).

Table 3: List of Damaging Translocations in a case of MM

	Gene	**Translocation**	**Chromosome and Position**	**Size (bp)**	**Zygosity**
Translocation	*RAG1*	Duplication	Chr11: 3536594776-36594997	221bp	Heterozygous
	KRAS	Duplication	chr12: 253684434-25368626	192bp	Heterozygous
	IGHV3-13 *IGH* *IGHV3OR16-10*	Inverted intra chromosomal translocation	Translocation from Chr14:106586315-106586759 To Chr16: 33006280-33006543	t(14;16) (q32.33;p11.2) 444bp	Homozygous
	LOC101929708 *IGKV1D-43* *IGK*	Inverted intra chromosomal translocation	Translocation from Chr22: 17365365-17385090 To Chr2:90249300-90249473	t(22;2) (q11.1;p11.2) 1925bp	Heterozygous
	DIS3	Intra-chromosomal reciprocal translocations	chr13:73348051-73348202	151bp	Heterozygous

DIS3, showed heterozygous 151bp duplication in exon 8 from chr13:73348051bp-73348202bp disrupting the reading frame (Table 3). However, an homozygous intra-chromosomal reciprocal translocations of 188bp between t(12;2), from chr12:7947173bp-7947361bp to chr12:8050850bp-8050956bp.

Identification of Secondary aberrations

Spectrums of secondary genomic mutations in the form of single base substitutions, small insertions-deletions, large duplications and deletions and translocations have been identified in several genes. Prioritizing the screening for those genes that are known to be secondary mutations led to identification of genes, *KRAS* and *DIS3*. *KRAS* showed a contiguous heterozygous duplication of a 192bp segment of exon 5 from chr12:253684434bp-25368626bp truncating the coding structure.

Table 4: List of secondary damaging mutations in a case of MM

Gene symbol	Chromosome No.	Position	Reference / Alternate base(s)	Protein sequence change	1k Genomes Allele Frequency	CHASM p-value	VEST p-value
CTDSP2	chr12	58220816	A>G	I106T	0	0.003	0.0028
		58220784	A>T	F117I	0	0.0128	0.0241
		58217422	T>C	D260G	0	0.0098	0.0245
		58217438	G>T	L255M	0	0.0038	0.0313
		58220841	C>T	D98N	0	0.008	0.0405
		58217774	C>A	K201N	0	0.0002	0.0053
		58220811	G>T	L108I	0	0.0084	0.0237
CTBP2	chr10	126683190	C>A	D750Y	0	0.017	0.0005
		126686659	G>A	R687W	0	0.0198	0.0021
		126683071	G>C	N789K	0	0.0278	0.0049
ADGRA3	chr4	22389848	T>C	N1149S	0	0.0176	0.0323
RFWD2	chr1	175958570	A>C	I592S	0	0.017	0.0111
FRMD4B	chr3	69267504	G>A	P253L	0	0.0258	0.0082
PSKH2	chr8	87076474	T>C	Y191C	0.0001996	0.0084	0.0239
ADAMTS3	chr4	73181637	G>T	P513T	0.0071885	0.0208	0.0298
TBC1D7	chr6	13321279	C>A	R81L	0.0005990	0.0362	0.0201
NOTCH1	chr9	139401802	C>A	D1200N	0.0001996	0.038	0.0231
CUBN	chr10	17087043	A>G	L1212S	0	0.0394	0.0365
CNGA1	chr4	47939651	C>A	R356I	0.0003993	0.0394	0.0268
		47939480	C>T	R413H	0.0037939	0.0228	0.0248
CDC27	chr17	45234481	T>C	R214G	0	0.0218	0.0121
SETD8	chr12	123892186	T>C	L332P	0	0.0002	0.0022
PABPC1	chr8	101721692	G>A	P414S	0	0.0278	0.0172
IGSF3	chr1	117156459	C>T	D254N	0	0.0284	0.0139
PCMTD1	chr8	52733242	C>T	R248H	0	0.0062	0.0325

Additionally, risk damaging polymorphisms and variants are listed in Tables 4 and 5 according to pathogenicity impact and cancer diver impact from CRAVAT toolkits. Sixteen genes including *NOTCH1, IGS3, CTBP2, CDC27, ADAMTS3, CTDSP2* and more listed genes found to carrying damaging mutations (Table 4). In Table 5 the DNA variants with high frequency and amino acid changes from higher to lower in number of variant are listed according to CRAVAT toolkit prioritizing of mutations in the case under study.

Table 5: List of variants in mutated genes in a case of MM

Chromosome	Gene Name	No. of Variants
chr11	*MUC6*	100
	OR8U1	66
	MUC5AC	62
	OR4C5	48
	OR4C3	33
	MUC5B	29
chr13	*PABPC3*	32
chr14	*AHNAK2*	31
chr16	*HYDIN*	46
chr17	*KCNJ12*	49
chr19	*MUC16*	97
chr2	*TTN*	64
chr3	*ZNF717*	166
chr5	*CMYA5*	30
chr6	*HLA-DQB1*	43
	HLA-DRB1	43
	HLA-A	33
	HLA-B	32
chr7	*MUC3A*	206
	MUC12	32

DISCUSSION

Over recent decades, treatment of myeloma cases have improved accomplished by the genetic profiling mechanisms and identifying mutations driving the transition of a normal plasma cell to end stage plasma cell leukemia. Understanding the co-evolution of the clones within their microenvironment impact is crucial for treatment of MM (Bianchi & Munshi, 2015). Because myeloma progression events follows Darwinian selection processes, the generation of a state associated with drug resistance, skip of apoptosis and an ability to continue proliferation independently of the bone marrow microenvironment happen during the course of their tumor formation (Pawlyn & Morgan, 2017). In current study with considering new manifest of mutations in MM based of large cohort studies and use of advanced technology we aimed to investigate a severe case of MM by WES to find out subsequent DNA mutations and aberrations for better treatment outcome. As our findings indicate translocation is initial event in this case in *IGH* and *IGK* loci because of the subject clinical reports which show the over expression of light chain immunoglobulin based on electrophoresis on M-spike protein test report. In support of this, the findings of genetic analyses of myeloma by using FISH technique and next generation sequencing in exome or whole genome level over the last quarter century have revealed approximately half of myelomas containing an immunoglobulin heavy chain (IgH) translocation (Barwick, *et al*., 2019).

On the other hand, for identifying driver mutations, we used CRAVAT a new web based tool for cancer genomic analysis that provided an end to end mutations workflow in cancer genome data. CRAVAT predict and prioritizing functional impact of list of mutations from tumor sequenced data and map to transcript to identify type of changes (missense, silent, etc) for predicting possible driver mutations. Analysis of WES data of this severe case of MM shows that several driver mutations are responsible for progression of MM, such as, *KMT2C (MLL3*), *BCL2, NOTCH2* and *DIS3* are the main driver gene mutations in this MM case. It suggests that increased number of driver mutations is associated with poor outcome for treatment in cancer cases. Unfortunately the subject received three cycles of radiation treatment and four cycles of chemotherapy over seven years. Also Bortosomib a proteosom inhibitor and MM panel drugs as common drug for MM cases practiced for him. The subject has a poor disease prognosis and his treatment is still continued with poor improvement. It indicates that heterogeneity in mutation level in this case is big challenge for finalizing treatment strategy.

CONCLUSION

Although MM, the second most common type of hematological malignancy still is an incurable form of cancer, but by favor of new sequencing technologies and target therapies, survival is improving, and newly diagnosed patients are now projected to live for more than five years in recent years. Therefore, based on DNA mutations and genomic aberrations in each patient individual treatment strategies should change for better outcome and more benefit for patients. As our findings indicate list of mutations are found in this severe case of MM that could be considered as key for target therapy treatment strategy for better outcome. We concluded that new target therapy drugs and monoclonal antibodies are the best options for practicing in this case based on to our WES data findings. At the end we suggest that precision medicine tailored to the genomic profile of each cancer patients would change the paradigm of cancer treatment especially in case of MM in close future.

Ethics approval and consent to participate
Prior to start of this study was approved by the Institutional Human Ethics Committee (IHEC-UOM No. 147/Ph.D/016/17) of University of Mysore. Written informed consent was obtained from case.

Consent for publication
Not applicable

Availability of data and materials
The datasets used during the current study are available from the first author.

Competing Interest
There is no competing of interest in the present study.

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Authors' contributions
Sample collected in hospital according to result of various clinical multiple myeloma diagnostic tests and proved by oncologist (second author). Whole Exome Sequencing data were analysed by different toolkits under guidance of third author. All authors have read and approved the manuscript.

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