Immunogenetics: Open Access

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Research Article

The Possible Impact of Glycosylation on the Binding of Ligand/Receptor and Activation of the NOTCH2 in Multiple Myeloma

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ABSTRACT

Background: Multiple Myeloma (MM) also is known as malignancy of plasma cells, is still incurable disease. In recent decades, advanced technologies such as next-generation sequencing methods, helped researchers to identify molecular players and driver mutations in MM. According to new and unprecedented findings, a series of drugs have been developed against MM for better treatment outcomes. The NOTCH signaling pathway is possible candidates for a novel therapeutic approach and emerging biomarkers and target pathway for drug designing in the case of MM.

Methods: We performed WES in four cases of MM and targeted point mutations in the NOTCH family and compared them with existing online data.

Result: It has been proved that the over activation of NOTCH2 is involved in the progression of MM. Also, it is clear that the extracellular domain of NOTCH2 protein is highly sensitive and ligand binding site is critical for activation of the NOTCH pathway.

Conclusion: We found a mutation in the glycosylation site of the extracellular domain of NOTCH2, and it seems it will be a possible candidate gene for further studies for target therapy and developing a new drug for MM.

Keywords: Multiple myeloma; Next generation sequencing; Target therapy; Drug designing

INTRODUCTION

Multiple myeloma (MM) is one of the plasma cell diseases characterized by clonal proliferation in the bone marrow with the presence of monoclonal protein in the blood or urine [1]. The disease usually starts and ranges from asymptomatic monoclonal gammopathy of unknown significance (MGUS) to smoldering multiple myeloma (SMM) to the malignant plasma cell leukemia [2].

In recent decades studies have identified several hundreds of mutations and DNA abrasions as key drivers for initiating and proliferation of MM cells. According to the findings of several studies, genes belong to Ras/MAPK, phosphatidyl/inositol3-kinase/Akt (PI3K/Akt), NOTCH, wingless (WNT), and nuclear factor-kappa B (NF-κB) pathways and mutations in cascade genes of these pathways contribute towards sustained activation in MM cells [3]. Among disrupted pathways in MM, NOTCH signaling also reported in many cases. The Notch signaling pathway is conserved evolutionarily and plays an essential role in the cell-cell communication process in many cell types and developmental processes [4]. Notch families are single-pass transmembrane proteins that function both as cell surface receptors and nuclear transcriptional regulators. There are four Notch receptors (Notch 1-4) in mammals [5]. Aberrant notch function has been associated with several human diseases and cancers. Cell-Cell interaction is an important event for the regulation of the Notch signaling pathway, also associated with the progression of different cancers [6].

The Notch pathway dysregulation in MM is due to the hyperexpression of Notch receptors and Jag1/2 ligands. This alteration affects MM cell biology and improves the ability of MM cells to shape the bone marrow (BM) niche, including a supportive behavior that promotes tumor progression [7]. MM cells are dependent on the BM niche that aid tumor growth and progress through adhesion molecules and soluble mediators, like interleukin-6 [8]. Consequent to the progression from MGUS to MM, the expression of Notch 1 and Jagged 1 increased sequentially. C-MAF (transcription factor) and MAFB (transcription factor) are responsible for NOTCH 2 expression and, Jagged 2 dysregulation is by promoter hypomethylation [9].

In the Notch family, several mutations were identified in receptors and ligands, causing different disorders such as alagille syndrome,

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Received: February 10, 2021, **Accepted:** February 25, 2021, **Published:** March 04, 2021

Citation: Abbaspourkharyeki M, Anvekar NJ, Ramachandra NB (2021) The Possible Impact of Glycosylation on the Binding of Ligand/Receptor and Activation of the NOTCH2 in Multiple Myeloma. Immunogenet OpenAccess. 6: 141.

spondylocostal dysostosis, tetrology of fallot, aortic valve disease [10]. Dysregulation of the Notch signaling pathway reported in several cancers, which make it potential for developing new anti-cancer drugs for target therapy and personalized medicine [11-13]. Despite most of the studies on NOTCH family focused on changes of expression pattern in this signaling pathway and impact on the activation of other genes leads to tumor formation in MM and many other cancers, there are few studies reported point mutations of NOTCH family in MM. In the current study, we performed whole exome sequencing (WES) investigations in four clinical cases of MM. We targeted all four NOTCH families to identifying damaging mutations by using predicting toolkits. Also, the lists of mutations identified in the NOTCH family were compared with existing reported data in different studies. These mutations were found to be involved in oncogenic pathways contributing to activation in MM cells.

MATERIALS AND METHODS

Samples selection

All four cases of MM for the current study were diagnosed by clinicians based on different clinical tests and MIR CT scanand were collected from Narayana Multispeciality Hospital in Mysuru, India. Diagnosis of MM required the presence of one of these markers like hypercalcemia, renal insufficiency, anemia, bone lesions. When there is malignant plasma cell, subsequently overproduction of a single antibody, resulting in a "spike" on the normal distribution, which is called an M spike. All four cases included in the current study diagnosed based on these clinical test results (Table 1). Cases with haematological or any other disorders excluded.

Table 1: Detail of all four cases with their clinical reports.

Samples	Sex	Age	Year of diagnosis	Clinical test and findings
MM-01	Male	47 yrs	2010	MIR-CT scan M-spike protein Bone Lesion
MM-02	Male	55 yrs	2018	MIR-CT scan M-spike protein Bone Lesion
MM-03	Female	62 yrs	2018	MIR-CT scan M-spike protein Bone Lesion
MM-04	Female	54 yrs	2016	MIR-CT scan M-spike protein Bone Lesion

Written informed consent was obtained from all four cases. The study protocol received ethical approval from the Ethics Committee of the University of Mysore (IHEC-UOM No. 147/Ph.D/016/17). A total of 5 ml of peripheral blood was collected from all the four cases in EDTA tubes for whole exome sequencing.

Whole exome sequencing

Whole exome sequencing was done at the coverage of 100x by using Illumina HiSeq 2500 system. The FASTQ file was aligned against the hg19 build of the human reference genome on Strand-NGS. For aligning the exome sequences, Strand-NGS v3.3.1 [14] was used in the current study due to its accuracy in terms of percent correctly mapped reads and receiver operating curves. Post alignment quality check was performed to remove bad variants. Damaging gene mutations were identified by subjecting variants into CRAVAT annotating program. CRAVAT is Cancer-Related Analysis of VAriants Toolkit, a program that currently employs CHASM and other analysis tools, and predicts the functional significance of mutations observed in the genomes of cancer cells [15]. CHASM-3.1 stands for "Cancer-specific High-throughput Annotation of Somatic Mutations" is the most updated version and predicts the damaging functional significance of somatic missense mutations observed in the cancer cells genome data. Also allowing mutations to be prioritized in subsequent functional studies, based on the probability that they give the cells a selective survival advantage [16,17].

RESULTS

The whole exome sequence data of four clinical cases of MM were revealed the presence of somatic mutations in the four genes of Notch1, Notch2, Notch3, and Notch4 under study. We found missense protein-coding alterations inNotch2 in all four cases (Table 1). Notch1 is located in chromosome 9, Notch2 in chromosome 1, Notch3 in chromosome 19, and Notch4 in chromosome 6 in humans. All the synonymous mutations excluded and nonsynonymous mutations listed in Table 1. Except for Notch2, we did not find any significant damaging mutations in other NOTCH families based on the CHASM scoring prediction toolkit (Table 1). In the first sample, all Notch (1-4) were found carrying the mutation, especially in Notch2 three missense mutations in exon one (g.120611964C>G, p.C19W) and 2 point mutations in the second exon (g.120572572C>T, p.E38K and g.120572547T>C, p.N46S) were found (Table 1). Mutations in Notch2 in the second sample were the same as that of the first sample, but we found a frameshift deletion in position g.120612003GG>- - (p.P6*). The third sample was also carrying a mutation in Notch2 (p.N46S).

Structure of Notch2

The structure of Notch2 consists of a single-pass transmembrane protein with extracellular domain and intracellular domain. The extracellular domain is related to binding the Notch ligands, and the Notch intracellular domain (NICD), act as a transcription factor, is responsible for transferring the Notch signal into the nucleus [18-30] and activating the Notch signal [21] .

p.N46S mutation in Notch2

Extracellular domain plays an essential role in the activation of Notch signaling due to binding of ligand and subsequent cleavage of the heterodimer (HD) and releasing the NICD that acts as a transcription factor for activation of specific genes and particular pathways [22]. In the typical structure of the extracellular domain 46th position, which is the position of Asparagine residue is the target for glycosylation, as reported in the uniprot protein database. Guiding of proper binding of ligand and its receptor is one of the crucial roles of glycosylation in cellular mechanisms [23] as we listed in Table 1 mutation in Notch2 (N46S) that leads to a change of asparagine to serine found in all four cases of MM.

DISCUSSION

Dysregulation of NOTCH signaling in MM could be described as a result of the overexpression of both receptors and ligands. In particular, immunohistochemical analyses revealed that Notch1, Notch2, and Jagged1 are highly expressed in primary

MM cells compared to low/undetectable levels in non-neoplastic counterparts [24]. Furthermore, the overexpression of NOTCH1, NOTCH2, and Jagged1 was reported upon disease progression from asymptomatic stage MGUS to MM [25]. In another study group of MM patients (approximately 6%) who carrying the translocations t(14;16)(q32;q23) and t(14;20)(q32;q11) NOTCH2 gene expression levels and activity were reported to be increased [26].

There are two ways of the Notch activation mechanism in cell-cell communications. In cis-inhibitory ligand can be expressed on the same cell surface as the receptor resulting in a cis inhibitory interaction that limits Notch activity. On the other hand, protein cross-talking between two neighbouring cells and binding of ligand/receptor, promoting the activation of Notch signaling, is known as trans-activation [27]. According to findings of a variety of experimental data, these two modes of interaction are well established, but the molecular basis for activating and inhibitory complexes is poorly understood. It has been proved that notch trans-activation promoted by Notch EGF11-12 and the DSL domain residues interactions [28].

In contrast, a study showed that, for cis-inhibition, the Notch EGF10-12 region is involved [29]. The initial processes leading to the activation of the Notch pathway upon ligand binding are undefined. Still, it is not clear that receptors activated solely by mechanotransduction initiated by ligand endocytosis and do a series of conformational changes lead to an allosteric effect. In the latter study, ligand binding could be rescued by the introduction of a calcium-binding mutation into EGF11, which uncouples the EGF10-11 interface [28]. It is possible, therefore, that changes in glycosylation state could alter the conformation of the receptor/ligand and their ability to cluster, which in turn would modulate binding and activation [29]. The impact of O-glycosylation on the binding of ligand/receptor and activation of the Notch pathway is poorly understood.

Furthermore, Notch2 is a crucial receptor for B-cell functions [30]. MM cells derived from B-cells in bone marrow and maturation in progenitor cells to adult cells involves in different cell signaling communications and activation of different pathways. However, the studies have shown that the high-resolution structures for individual parts of the receptor, ligand, and transcriptional complex, suggesting that a complete molecular description is a realistic prospect [31].

CONCLUSION

The result of the study suggests that large cohort analysis with more number of MM cases and controls needed to study the possible role of Notch2 (N46S) mutation that is the site of glycosylation in protein, how changes function of receptor/ligand binding and finally to find out the possible role in the progression of MM. In addition, conducting proteomics studies of O-glycosylation, receptor endocytosis, and structural biology could allow a molecular dissection of the activation mechanism and its regulation by post-translational modification and, as a consequence, offer new targets for developing new anti-cancer drugs especially for MM cases.

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