HLA-DPA1 gene is a potential predictor with prognostic values in multiple myeloma

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Research article

Keywords: multiple myeloma, hypoxia, prognosis, bioinformatics analysis

DOI: https://doi.org/10.21203/rs.3.rs-36641/v1

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Abstract

**Background:** Multiple myeloma (MM) is an incurable hematological tumor, which is closely related to hypoxic bone marrow microenvironment. We took integrated bioinformatics analysis with expression profile GSE110113 downloaded from National Center for Biotechnology Information-Gene Expression Omnibus (NCBI-GEO) database, and screened out major histocompatibility complex, class II, DP alpha 1 (HLA-DPA1) as a hub gene related to hypoxia in MM.

**Methods:** Differentially expressed genes (DEGs) were filtrated with R package “limma”. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were performed using “clusterProfiler” package in R. Then, protein-protein interaction (PPI) network was established. Hub genes were screened out according to Maximal Clique Centrality (MCC). PrognoScan evaluated all the significant hub genes for survival analysis. ScanGEO was used for visualization of gene expression in different clinical studies.

**Results:** HLA-DPA1 was finally picked out as a hub gene in MM related to hypoxia. MM patients with down-regulated expression of HLA-DPA1 has statistically significantly shorter disease specific survival (DSS) (COX \( p =0.005411 \)). Based on the clinical data of GSE47552 dataset, HLA-DPA1 expression showed significantly lower in MM patients than that in healthy donors (HDs) (\( p=0.017 \)).

**Conclusion:** We identified HLA-DPA1 as a hub gene in MM related to hypoxia. HLA-DPA1 down-regulated expression was associated with MM patients’ poor outcome. Further function and mechanism studies are need to investigate HLA-DPA1 as potential therapeutic target.

**Background**

Multiple myeloma (MM) is a hematological malignancy which is characterized by aberrant plasma cells infiltration in the bone marrow and complex heterogeneous cytogenetic abnormalities[1]. Accumulation of abnormal plasma cells replace normal hematopoietic cells and leads to “CRAB” - hypercalcemia, renal failure, anemia, and bone lesions, even fetal outcome eventually[2]. With the deepening of basic and clinical research, novel drugs mainly including proteasome inhibitors and immunomodulatory drugs have improved patients’ outcome to some extend[3, 4]. Besides, high-dose chemotherapy and tandem autologous stem cell transplant (ASCT), together with supportive care have significantly prolonged patients’ progression-free survival (PFS) and overall survival (OS)[5]. However, MM remains an uncurable disease as underlying molecular mechanisms of pathogenesis and progression are still largely unclear. Quite a few patients cannot get diagnosis and proper treatment in time. Therefore, identifying key mechanisms regulating MM is critically important for early diagnosis and targeted therapy.

With the advances of high-throughput platforms and microarray, more and more molecular heterogeneity on MM has been recognized[6, 7]. Hypoxia plays an important role in occurrence and development of MM[8, 9] and more related pathogenesis is still urgent needs to be explore for better diagnosis and treatment. In order to find potential biomarker of MM related to hypoxia, we analyzed the differentially
expressed genes (DEGs) function and pathways between normoxia and hypoxia-resistant (HR) MM cell lines contained in GSE110113 dataset. Major histocompatibility complex, class II, DP alpha 1 (HLA-DPA1) was screened out as a hub gene associated with poor outcome of MM related to hypoxia. In addition, survival analysis and gene expression level were visualized with online clinical data. HLA-DPA1 is a potential biomarker for MM and more research needs to be performed.

Materials And Methods

Data source and DEGs identification

Gene expression profile GSE110113 was downloaded from National Center for Biotechnology Information-Gene Expression Omnibus (NCBI-GEO) database (https://www.ncbi.nlm.nih.gov/geo/)[10]. The array data of GSE110113 is based on GPL6244 platform (HuGene-1_0-st Affymetrix Human Gene 1.0 ST Array). There are four parental cells (RPMI8226, KMS-11, U266, IM-9) and four HR cells that derived from above parental cells. Two group cells were cultured under normoxic condition (20% O\textsubscript{2}) and hypoxic condition (1% O\textsubscript{2}) for 24hours, respectively.

R package “limma” was used to identify DEGs between normoxic and HR cells groups[11]. The screen criteria are adjusted \( p \) value < 0.05 and \([\log2\text{FoldChange (log2FC)}]\) > 1. All genes were visualized by volcanic maps and top 50 dramatically altered genes were selected to draw a heatmap by R package “ggplot2”[12].

GO and KEGG analysis

Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were conducted by R package “clusterProfiler”[13] which is for functional classification and gene clusters enrichment. GO enrichment includes biological process (BP), molecular function (MF), and cellular component (CC) three subontologies. Analysis results were displayed with “GOplot” package of R[14]. In addition, relationship between pathways was further analyzed with the ClueGO plug-ins of Cytoscape software 3.7.2[15]. Threshold of the analysis was \( p \) value < 0.05.

PPI network analysis

To clarify the relationships of proteins encoded by selected enrichment genes, a protein-protein interaction (PPI) network was established using the STRING database (https://string-db.org)[16]. Cytoscape software 3.7.2 was used to visualize the genes with minimum interaction score more than 0.4[15]. Then, we utilized cytoHubba plug-ins to recognize interaction degree of hub-gene clustering according to the Maximal Clique Centrality (MCC) methods. Wayne diagram produced by online tool Bioinformatics & Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to show the overlapped genes.

Survival analysis
To assess the prognostic value of selected genes in MM patients, survival analysis was performed with the PrognoScan database ([http://dna00.bio.kyutech.ac.jp/PrognoScan/](http://dna00.bio.kyutech.ac.jp/PrognoScan/))[17]. Based on GSE2658 dataset (n=559) provided by Zhan[18], relationship between gene expression and corresponding disease specific survival (DSS) were researched. Besides, according to online ScanGEO database ([http://scangeo.dartmouth.edu/ScanGEO/](http://scangeo.dartmouth.edu/ScanGEO/))[19], we found GSE47552[20] and GSE2113[21] datasets which involving HLA-DPA1 expression compared to different degree of disease progression and healthy donors (HDs). Gene expression level in clinical patients was explored with the two datasets.

**Results**

**Identification of DEGs**

This study was performed as a multiple strategy to pick out the hub gene related to hypoxia in MM dataset GSE110113. The hub gene was then validated with online clinical data (Figure 1). Myeloma cells were divided into normoxic and HR groups. 1285 DEGs (614 up-regulated and 671 down-regulated genes) were screened out using “limma” R package (Figure 2A) and a heatmap depicted top 50 genes (Figure 2B).

**GO and KEGG enrichment analysis**

All DEGs were performed GO and KEGG enrichment analysis to further explore their functions with R package “clusterProfiler”. Three subontologies including BP, MF, CC were examined in GO analysis. Adaptive immune response pathway ($p=1.31e-10$, FDR=6.59e-07), cell adhesion molecule binding pathway ($p=0.000162$, FDR=0.104) and receptor complex pathway ($p=1.23e-05$, FDR=0.00221) were selected as the most significant pathway in each subontologies, respectively (Figure 3A-C). Then, adaptive immune response pathway was picked out. KEGG enrichment analysis with R package “clusterProfiler” screened out herpes simplex virus 1 infection pathway ($p=1.39e-08$, FDR=3.63e-06) (Figure 3D). Further analysis of KEGG result was performed with ClueGO to identified the interrelation of pathways. Herpes simplex virus 1 infection pathway remained the most significant pathway associated with DEGs (Figure 3E, F). 65 and 70 DEGs were involved in the two selected pathways, respectively (Table 1). Next, we identified 9 common genes by overlapping DEGs in the two pathways with Wayne diagram (Figure 3G). They were SYK, POU2F2, LTA, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DMA and HLA-DMB.

**PPI network**

To find and understand the hub genes, we constructed a PPI network consisting of all the DEGs from the two most significant pathways mentioned above in STRING (Figure 4A, B). We used Cytoscape plug-ins cytoHubba to screen top 15 candidate hub genes according nodes rank (Figure 4C, D) and they were listed in Table 2. Subsequently, we identified 3 common genes, HLA-DPA1, DQHLA-DQA1 and HLA-DQB1 of the two pathways as candidate hub genes.
**Survival analysis**

Finally, we identified the correlation between candidate hub genes and the prognosis of patients with MM. Potential prognostic value of candidate hub genes were assessed with PrognoScan. As a result, only HLA-DPA1 (Cox \( p=0.005411 \)) was statistically significant associated with DSS of MM patients based on GSE2658 dataset covering 559 patients (Figure 5A, Additional file 1). In addition, ScanGEO exploration results showed HLA-DPA1 gene expression is significant lower in MM patients compared to HDs \( (p=0.017) \) in GSE47552(Figure 5B). GSE2113 dataset contains 7 monoclonal gammopathy of undetermined significance (MGUS), 39 newly diagnosed MM and 6 plasma-cell leukaemia (PCL) patients. As the severity of the disease increased, the level of HLA-DPA1 gene expression gradually decreased \( (p=0.007) \) (Figure 5C). Further verification of this gene in clinical research remains need.

**Discussion**

In this study, we analyzed DEGs between normoxic and hypoxic cultured MM cells based on GSE110113 dataset. Enrichment analysis picked out adaptive immune response pathway and herpes simplex virus 1 infection pathway. It is well-known that human immune system can eradicate cancer cells. Cancers’ occurrence and development is critical associated with immune response adaptation and immune escape which have been demonstrated with mice model[22, 23]. Herpes simplex virus (HSV) 1 has antitumor effect which mainly depends on its cytotoxic effect and replication ability with tumor in order to produce more virus for tumor lysis[24]. Previous study indicated HSV was associated with occurrence of MM and Bortezomib could inhibit HSV infection by halting viral capsid transport to the nucleus[25].

Next, establishment of PPI network and further analysis with Cytoscape plug-ins cytoHubba identified 3 candidate hub genes, HLA-DPA1, HLA-DQA1 and HLA-DQB1. The major histocompatibility complex (MHC) class II proteins include HLA-DR, HLA-DQ and HLA-DP classical proteins and they only expressed on professional antigen-presenting cells (B lymphocytes, dendritic cells and macrophages) to activate CD4+ T cells[26]. They could participate in cancer development as it has been proved that dysregulation of immune function which involved antigen presentation was associated with cancer[27]. Subsequently, survival analysis revealed HLA-DPA1 as hub gene associated with survival of MM patients, and gene expression of HLA-DPA1 was significantly lower compared to HDs and MGUS.

Hypoxia is common and essential in various cancers which can bring different gene expression change during metabolic adaptations[28]. As a result, cancer cells can survival and keep high rate proliferation. Previous studies have shown hypoxic bone marrow microenvironment play a critical role in MM through different aspects. For instance, endothelial cells (ECs) in MM patients having a hypoxic phenotype could keep up with enhanced angiogenesis in cancer growth and metastasis[8]. Hypoxia induced MM cells dedifferentiation, stem-cell like state acquisition without apoptosis and increased drug resistance to proteasome inhibitors[9].

HLA-DPA1, also known as HLA-DP1A, HLASB or DPA1, belongs to the HLA class II alpha chain paralogues[29]. As a result, HLA-DPA1 function as an MHC class II receptor to participate in immune
response and antigenic peptides presentation. Clinical study on adrenocortical tumors (ACT) indicated low expression of HLA-DPA1 was associated with poor prognosis[30]. Acute myeloid leukemia (AML) relapse after transplantation was analyzed by Christopher MJ et al. It was proved to be associated with dysregulation of pathways which had an influence on immune function. HLA-DPA1 and several other MHC class II genes’ down-regulation were involved as they function in antigen presentation[31]. Other several researches showed MHC class II genes had crucial relationship with cancer immunology, and down-regulation of related genes indicated a poor prognosis[27, 32, 33].

**Conclusion**

HLA-DPA1 was screened out as a hub gene related to hypoxia in MM. Down-regulated expression of HLA-DPA1 was associated with shorter survival time of MM patients. Notably, 3 candidate hub genes before survival analysis were all related to immune response. According to the result, more researches on immune process of MM pathogenesis can help us to better understand MM. This study provided a novel insight into HLA-DPA1 as a critical potential biomarker for MM.

**Tables**

*Table 1: DEGs identified from selected pathways of GO and KEGG.*

| DEGs                                    | Gene names                                                                 |
|-----------------------------------------|---------------------------------------------------------------------------|
| **Adaptive immune response pathway**    | ADA, ADCY7, CD8B, DENND1B, EMP2, FAM49B,                                  |
|                                         | IGKV1D-8, LAIR1, PYCARD, SMAD7, SYK,                                      |
|                                         | THEMIS, TLR4, TNFRSF1B, TNFRSF21, ULBP3,                                  |
|                                         | UNC93B1, ZP3, BATF, C2, CAMK4, CD274, CD48,                               |
|                                         | CD70, CD79A, CD79B, CD80, CD86, CEACAM1,                                 |
|                                         | CTSH, ERAP2, GPR183, HAVCR2, HLA-DMA, HLA-DMB,                            |
|                                         | HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, ICAM1, IL23A, IL23R, INPP5D,      |
|                                         | JAK3, LAMP3, LILRB4, LTA, MEF2C, NFKBIZ, PAG1,                            |
|                                         | POU2F2, PTPRC, RAB27A, RORA, SAMSIN1, SASH3, SLAMF1, SLAMF6, SLAMF7,     |
|                                         | SPN, TEC, TFRC, TNFAIP3, TNFSF13B,                                       |
|                                         | TXK                                                                       |

| **Herpes simplex virus 1 infection pathway** | CCL2, IKBKE, SYK, TNFRSF1A, ZNF26, ZNF382, ZNF605, ZNF717, BIRC3, CHUK, EBF2AK3, HLA-DMA, HLA-DMB, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, IFIH1, IRF9, LTA, OAS1, OAS2, OAS3, POU2F2, SP100, STAT1, ZFP30, ZFP82, ZNF100, ZNF155, ZNF175, ZNF208, ZNF221, ZNF222, ZNF223, ZNF234, ZNF254, ZNF256, ZNF283, ZNF30, ZNF404, ZNF415, ZNF429, ZNF43, ZNF431, ZNF439, ZNF45, ZNF486, ZNF510, ZNF543, ZNF546 |
Table 2: The top 15 genes with the highest score of each pathway through the Cytoscape “cytoHubba” module analysis.

| Rank | Name       | Score | Name       | Score |
|------|------------|-------|------------|-------|
| 1    | PTPRC      | 11394 | IRF9       | 40560 |
| 2    | CD86       | 9512  | OAS1       | 40560 |
| 3    | ICAM1      | 9390  | OAS2       | 40560 |
| 4    | CD80       | 9146  | OAS3       | 40560 |
| 5    | TNFSF13B   | 5760  | SP100      | 40440 |
| 6    | TLR4       | 5337  | HLA-DQB1   | 40440 |
| 7    | CD274      | 4108  | HLA-DQA1   | 40440 |
| 8    | SPN        | 3648  | HLA-DPB1   | 40440 |
| 9    | HLA-DQA1   | 3528  | HLA-DPA1   | 40440 |
| 10   | CD70       | 2880  | STAT1      | 250   |
| 11   | HLA-DQB1   | 2808  | IFIH1      | 126   |
| 12   | SYK        | 2410  | HLA-DMB    | 120   |
| 13   | HLA-DPA1   | 1992  | HLA-DMA    | 120   |
| 14   | CD48       | 1566  | TNFRSF1A   | 12    |
| 15   | TNFRSF1B   | 1493  | CCL2       | 10    |

**Abbreviations**

MM: multiple myeloma

NCBI-GEO: National Center for Biotechnology Information-Gene Expression Omnibus

HLA-DPA1: major histocompatibility complex, class II, DP alpha 1

DEGs: differentially expressed genes
GO: Gene Ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes
PPI: protein-protein interaction
MCC: Maximal Clique Centrality
DSS: disease specific survival
HDs: healthy donors
ASCT: autologous stem cell transplant
PFS: progression-free survival
OS: overall survival
HR: hypoxia-resistant
log2FC: log2FoldChange
BP: biological process
molecular function (MF)
CC: cellular component
MGUS: monoclonal gammopathy of undetermined significance
PCL: plasma-cell leukaemia
HSV: herpes simplex virus
MHC: major histocompatibility complex
ECs: endothelial cells
ACT: adrenocortical tumors
AML: acute myeloid leukemia

Declarations

Ethics approval and consent to participate: Not applicable.
Consent for publication: Not applicable.
Availability of data and materials: The dataset analysed during the current study are available in the NCBI-GEO repository, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE110113.

Competing interests: The authors declare that they have no competing interests.

Funding: This study was supported by Natural Science Foundation of Jiangsu Province for Youth (BK20180372), Jiangsu Provinical Medical Youth Talent (QNRC2016812), and Key Medical of Jiangsu Province (ZDXKB2016020).

Authors’ contributions: JY performed mainly data analysis and wrote the manuscript. FW performed part data analysis. B-A C conceived of and designed the study. All authors read and approved the final manuscript.

Acknowledgements: Not applicable.

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**Figures**
Figure 1

A schematic view of the procedure of the study with GSE110113.
Figure 2

Identification of differentially expressed genes in GSE110113 dataset. A Volcano plot of GSE110113 dataset. Red plots represent genes with adjusted p value < 0.05 and \([\log_{2}\text{FoldChange (log2FC)}] > 1\). Other plots represent the remaining genes with no significant difference. B Heatmap of the top 50 DEGs (50 up-and 50 down-regulated genes). DEGs, differentially expressed genes.
Figure 3

GO and KEGG enrichment analysis. A-D The bubble chart showed the top 10 pathways with significant difference. A The GO biological process enrichment analysis. B The GO molecular function enrichment analysis. C The GO cellular component enrichment analysis. D The KEGG enrichment analysis. E, F Interrelation analysis of pathways via assessment of KEGG processes in ClueGO. E The interrelation
between pathways of KEGG. F Numbers of genes enriched in the identified pathways. G Venn diagram showed the common gene of candidate genes. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Figure 4

PPI network analysis. A, B The PPI analysis at STRING. C, D Cytoscape plug-ins cytoHubba analysis of candidate genes after PPI analysis. A, C Genes identified from adaptive immune response pathway. B, D Genes identified from herpes simplex virus 1 infection pathway. PPI, protein-protein interaction.
Figure 5

Analysis of hub gene HLA-DPA1. A Kaplan-Meier survival curves comparing high and low expression of HLA-DPA1 in MM with PrognoScan. B, C HLA-DPA1 gene expression in different clinical datasets. B HLA-DPA1 gene expression in GSE47552 dataset. C HLA-DPA1 gene expression in GSE2113 dataset.

Supplementary Files

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