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# Identification of genes and pathways involved in malignant pleural mesothelioma using bioinformatics methods

Xingsheng Liu, Kun Qian, Gaojun Lu, Peng Chen and Yi Zhang\* 

## Abstract

**Background:** Malignant pleural mesothelioma (MPM) is a rare tumor in the pleura. This study was carried out to identify key genes and pathways that may be involved in MPM.

**Methods:** Microarray datasets GSE51024 and GSE2549 were analyzed for differentially expressed genes (DEGs) between normal and MPM tissues. The identified DEGs were subjected to functional analyses using bioinformatics tools.

**Results:** A total of 276 DEGs were identified, consisting of 187 downregulated and 79 upregulated genes. Gene ontology and Kyoto encyclopedia of genes and genomes pathway enrichment analysis indicated that the DEGs were enriched in extracellular structure organization, extracellular matrix, and ECM–receptor interaction. Due to high degree of connectivity among 24 hub genes, EHZ2 and HMMR are likely to play roles in the carcinogenesis and progression of MPM. The two genes were found over-expressed in MPM tissues. Patients with elevated EHZ2 and HMMR expressions had poor overall survival.

**Conclusions:** EHZ2 and HMMR are identified to be the hub genes for MPM and they may be further characterized to better understand the molecular mechanisms underlying the carcinogenesis of MPM.

**Keywords:** Microarray, Gene expression profile, Cancer, Differentially expressed genes, Protein–protein interaction, Bioinformatics

## Background

Malignant pleural mesothelioma (MPM) is a rare and highly lethal neoplasm arising from the mesothelial cells lining the pleural cavity [1]. However, due to wide use of asbestos, its incidence has been dramatically increasing since the mid-twentieth century [2]. Among histological types (epithelial, sarcomatoid, and biphasic or mixed), the sarcomatoid carcinoma is particularly associated with a more aggressive progression [3]. Because of a lack of effective treatment of the disease, overall survival (OS)

of patients with MPM is around one year after diagnosis and up to two years after receiving intensive multimodal therapy [4]. Therefore, it is important to elucidate the molecular mechanisms underlying the carcinogenesis and progression of MPM.

During the past decade, tremendous progress has been made in elucidating the pathogenesis of MPM. For example, microarray technology has been used to examine the oncogenic genetic alterations in MPM, such as homozygous deletion of the 9p21 locus which harbors the p16/CDNK2A gene, germline mutations of BAP1 and mutations of multiple Hippo genes [5, 6]. Moreover, NAT2, a polymorphic gene encoding enzymes in xenobiotic and oxidative metabolism or involved in genome stability, is

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found to increase MPM risk in asbestos-exposed populations [7]. From epigenetic perspectives, SETD2 (SET domain containing protein 2, a histone methyltransferase), specifically H3K36me3 (histone H3 trimethylated at lysine 36), is identified as epigenetic regulator of gene expression associated with MPM [5]. Based on the methylation status of the human androgen receptor gene, MPM was found to have a polyclonal origin [8]. An exploratory analysis proposed that PI3K/Akt/mTOR signaling pathways and downstream proteins are frequently activated in MPM and can be used to provide prognostic information [9]. In addition, chronic inflammation also plays a key role in the pathogenesis of MPM as a result of asbestos exposure [10]. However, the molecular mechanisms underlying the disease are still largely unclear and more genes need to be identified to advance our understanding.

In the present study, two mRNA microarray datasets from Gene Expression Omnibus (GEO) databases were analyzed to identify the common DEGs between MPM and non-cancerous tissues. Enrichment analysis, network-based approaches and ONCOMINE data mining were applied to identify hub genes related to MPM. The findings would provide new insights into molecular mechanisms related to MPM pathogenesis and clues to develop diagnostic and therapeutic approaches for the cancer.

## Materials and methods

### Microarray data

The mRNA expression profiles were obtained from the GSE2549 and GSE51024 datasets [11]. The GSE2549 dataset contains 40 MPM samples and 4 non-cancerous tissue samples. The GSE51024 dataset has 55 MPM samples and 41 non-cancerous tissue samples (Table 1). The two datasets were downloaded from GEO (<http://www.ncbi.nlm.nih.gov/geo>) which is a public repository containing data obtained through high throughput gene expression assays, chips and microarrays. The data were uniformly pre-processed using the Robust Multichip Average algorithm for background correction, quantile normalization and log<sub>2</sub>-transformation [12]. The probes were converted into corresponding genes using the annotation information available from the gene platforms (GPL96-57554 and GPL570-55999, respectively).

### Identification of differentially expressed genes (DEGs)

The DEGs between MPM and non-cancerous tissue samples were identified using the limma package in R language. Adjusted P-values (adj. P) and false discovery rate were used to balance statistically significant genes and false-positives. The threshold of adj. P was set at <0.05 and |log<sub>2</sub>(fold-change)| > 1. Volcano figures were plotted to identify the DEGs in the two datasets using ggplot2 package (version 3.5.3). Venn diagram was then constructed to determine the common DEGs originated from both datasets.

### Construction of protein–protein interaction (PPI) network, module analysis and hub gene selection

The Search Tool for the Retrieval of Interacting Genes (STRING; <http://string-db.org>) (version 10.0) provides uniquely comprehensive interaction information of experimental and predicted proteins. In this study, the PPI network of common DEGs was constructed using STRING online database. A combined score >0.4 was considered as the reliability threshold for interaction. Cytoscape (version 3.4.0; [www.cytoscape.org](http://www.cytoscape.org)), an open source bioinformatics software platform, was used to visualize the molecular interactions in the PPI networks obtained with the STRING online databases. Subsequently, the module analysis of the PPI network was performed using Molecular Complex Detection (MCODE) (version 1.5.1), which is an APP for analyzing hub genes according to topology to find highly connected regions which had MCODE scores >5, degree cut-off = 2, node score cut-off = 0.2, Max depth = 100 and k-score = 2. Then, the hub genes were selected from the module based on both the degree of the connectivity and the node status.

### Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

GO is widely used to annotate genes and gene products, and to functionally characterize high-throughput genome or transcriptome data. KEGG are databases capable of interacting with genomes, biological pathways, diseases, drugs, and chemicals [13, 14]. GO and KEGG pathway enrichment analyses were carried out using clusterProfiler package and Signaling Pathway Impact Analysis (SPIA) (v3.4.0). The threshold was set

**Table 1** Details of MPM mRNA expression data collected from GEO data sets

| Author (year)        | Materials | Accession/ID | Platform     | Normal samples | MPM samples |
|----------------------|-----------|--------------|--------------|----------------|-------------|
|                      | Tissue    | GSE2549      | GPL96-57554  | 4              | 40          |
| Suraokar et al. [11] | Tissue    | GSE74190     | GPL570-55999 | 41             | 55          |

GEO Gene Expression Omnibus, GPL GEO platform, MPM Malignant Pleural Mesothelioma, mRNA messenger RNA

at  $P$ -value  $< 0.05$ . Enriched GO terms or KEGG signaling pathways were ranked based on the degree of connectivity of hub genes using GPlot package (version 1.4.0).

#### Validation of the selected hub genes in bioinformatic database

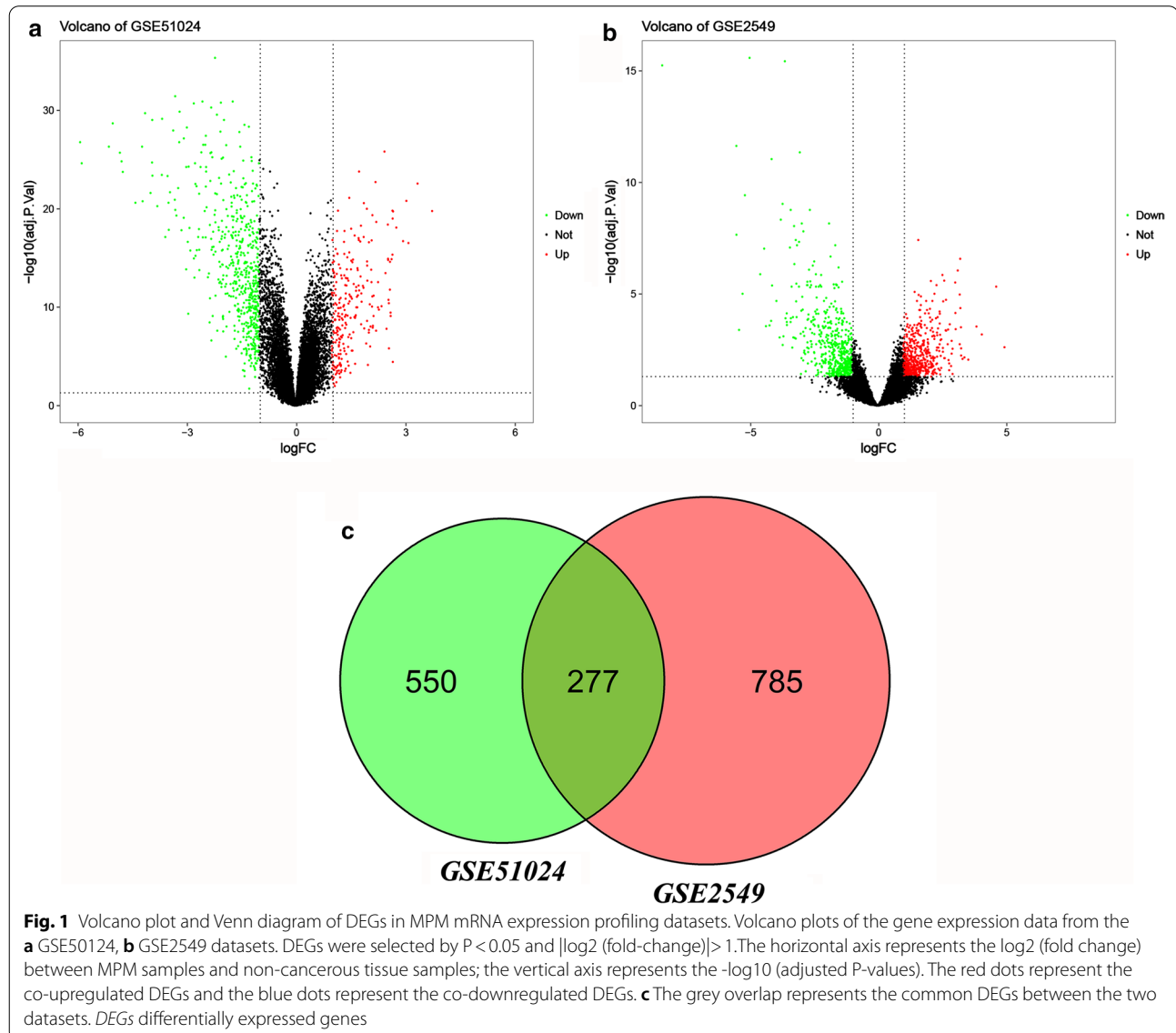
OncoPrint ([www.oncoPrint.org](http://www.oncoPrint.org); Ion Torrent; Thermo Fisher Scientific, Inc.) is an online cancer microarray database developed to facilitate the discovery of oncogenes through genome-wide expression analyses [15]. To validate the expression level of the identified hub genes in MPM, gene expression data in the OncoPrint database were analyzed with  $P$ -value set to  $< 0.05$ , thresholds for fold-change and gene rank set to 'all'. In order to calculate the prognostic significance of the selected hub

genes, Kaplan–Meier survival analyses were performed based on the clinical information from TCGA datasets using UALCAN, which is a comprehensive, user-friendly, and interactive web resource [16]. For this analysis, the patients were separated into high and low expression groups according to the median of the hub gene expression levels.

## Results

### Identification of DEGs

After standardization of the microarray results, a total of 827 and 1062 DEGs were identified in the GSE51024 and GSE2549 datasets, respectively. The volcano figures were plotted to visualize DEGs between MPM and non-cancerous tissue samples (Fig. 1a, b). The Venn diagram



showed that 276 DEGs were common in the two datasets, of which 187 and 79 genes were downregulated and upregulated in both datasets, respectively (Fig. 1c).

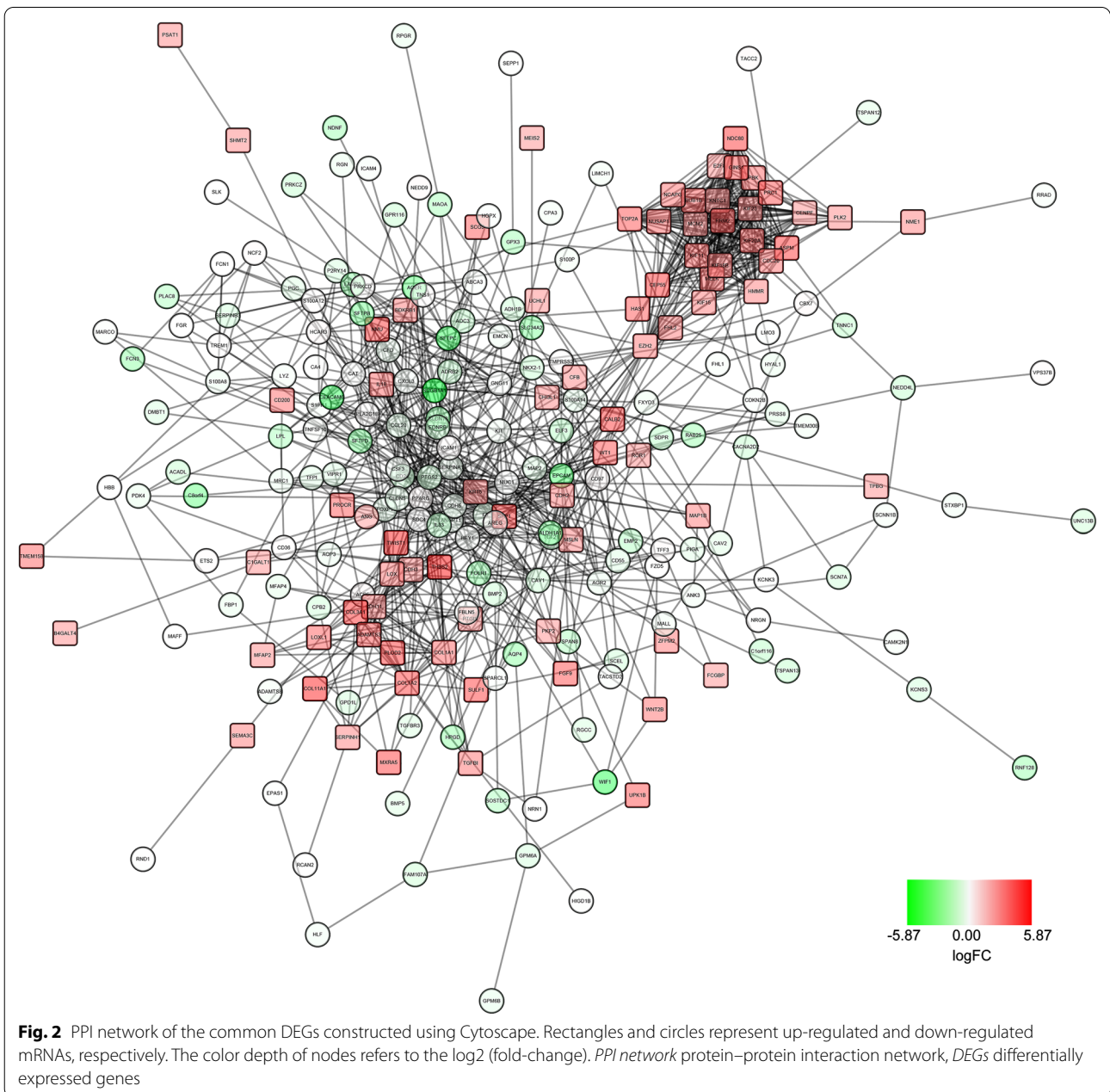
**Construction of PPI network and module analysis**

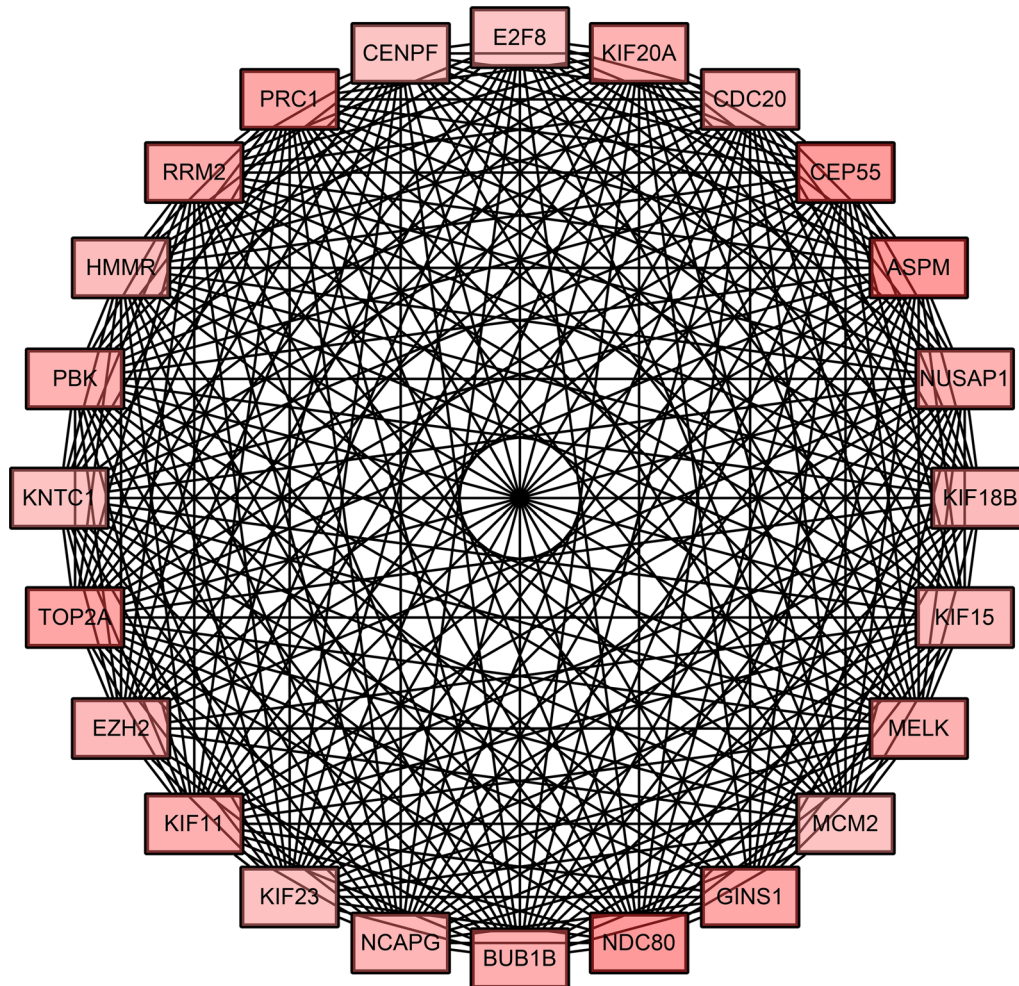
To investigate protein interactions, PPI network of the common DEGs was downloaded from STRING and the PPI network was constructed using Cytoscape. The network contained 239 nodes and 56,882 edges after removing isolated nodes (Fig. 2). And then, a module of significant hub genes was obtained from the PPI network

of DEGs using MCODE, consisting of 24 nodes and 552 edges (Fig. 3). The hub genes were obtained with a connectivity degree of  $\geq 19$  in the module. Considering the connectivity and the node status together, EZH2 and HMMR might play the most important roles in the carcinogenesis or progression of MPM (Table 2).

**GO and KEGG pathway enrichment analysis**

After GO and KEGG pathway enrichment analysis, the top 10 terms of each category ranked by adjusted P-value are presented in Fig. 4a. These results showed





**Fig. 3** A significant module of hub genes obtained from the PPI network of DEGs using MCODE. PPI network protein–protein interaction network, MCODE molecular complex detection

that the DEGs in biological process (BP) were mainly enriched in extracellular matrix organization, leukocyte migration, extracellular matrix organization, reproductive structure development, mesenchyme development, transmembrane receptor protein serine/threonine kinase signaling pathway, renal system development, mesenchymal cell differentiation, regulation of cell–substrate adhesion and extracellular matrix assembly. The DEGs in cellular component (CC) were mainly enriched in extracellular matrix, collagen-containing extracellular matrix, membrane raft, membrane microdomain, membrane region, apical plasma membrane, collagen trimer, extracellular matrix component, fibrillar collagen trimer, and banded collagen fibril, while the DEGs in molecular function (MF) were significantly enriched in extracellular matrix structural constituent, channel regulator activity, cargo receptor

activity, ion channel regulator activity, integrin binding, scavenger receptor activity, potassium channel regulator activity, signaling pattern recognition receptor activity, pattern recognition receptor activity, platelet-derived growth factor binding. KEGG pathway analysis revealed that the common DEGs were mainly enriched in Complement and coagulation cascades, AGE-RAGE signaling pathway in diabetic complications, ECM–receptor interaction and Malaria (Fig. 4b). The distribution of the common hub genes in the GO and KEGG pathway analysis is shown in Fig. 5. The hub genes in BP, CC and MF were mainly enriched in extracellular structure organization (Fig. 5a), extracellular matrix (Fig. 5b) and extracellular matrix structural constituent (Fig. 5c), respectively. In the KEGG pathways the hub genes were mainly located in ECM–receptor interaction (Fig. 5d).

**Table 2** Characteristics of 24 hub genes obtained from a significant module of the PPI network

| Name   | Full name   | Degree | Regulation | MCODE node status |
|--------|---|--------|------------|-------------------|
| EZH2   | Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit | 35     | Up         | Clustered         |
| KIF11  | Kinesin Family Member 11                                  | 27     | Up         | Clustered         |
| ASPM   | Abnormal Spindle Microtubule Assembly                     | 26     | Up         | Clustered         |
| HMMR   | Hyaluronan Mediated Motility Receptor                     | 25     | Up         | Seed              |
| CEP55  | Centrosomal Protein 55                                    | 25     | Up         | Clustered         |
| CDC20  | Cell Division Cycle 20                                    | 25     | Up         | Clustered         |
| BUB1B  | BUB1 Mitotic Checkpoint Serine/Threonine Kinase B         | 25     | Up         | Clustered         |
| PRC1   | Protein Regulator Of Cytokinesis 1                        | 25     | Up         | Clustered         |
| RRM2   | Ribonucleotide Reductase Regulatory Subunit M2            | 24     | Up         | Clustered         |
| TOP2A  | DNA Topoisomerase II Alpha                                | 24     | Up         | Clustered         |
| PBK    | PDZ Binding Kinase  | 24     | Up         | Clustered         |
| KIF20A | Kinesin Family Member 20A                                 | 24     | Up         | Clustered         |
| MCM2   | Minichromosome Maintenance Complex Component 2            | 24     | Up         | Clustered         |
| KIF23  | Kinesin Family Member 23                                  | 24     | Up         | Clustered         |
| NUSAP1 | Nucleolar And Spindle Associated Protein 1                | 24     | Up         | Clustered         |
| NCAPG  | Non-SMC Condensin I Complex Subunit G                     | 24     | Up         | Clustered         |
| KIF15  | Kinesin Family Member 15                                  | 23     | Up         | Clustered         |
| MELK   | Maternal Embryonic Leucine Zipper Kinase                  | 23     | Up         | Clustered         |
| NDC80  | NDC80 Kinetochore Complex Component                       | 23     | Up         | Clustered         |
| CENPF  | Centromere Protein F                                      | 22     | Up         | Clustered         |
| KNTC1  | Kinetochore Associated 1                                  | 22     | Up         | Clustered         |
| KIF18B | Kinesin Family Member 18B                                 | 21     | Up         | Clustered         |
| E2F8   | E2F Transcription Factor 8                                | 21     | Up         | Clustered         |
| GINS1  | GINS Complex Subunit 1                                    | 19     | Up         | Clustered         |

*MCODE node status* Molecular Complex Detection Node Status

### Validation of EZH2 and HMMR expression and their prognostic significance

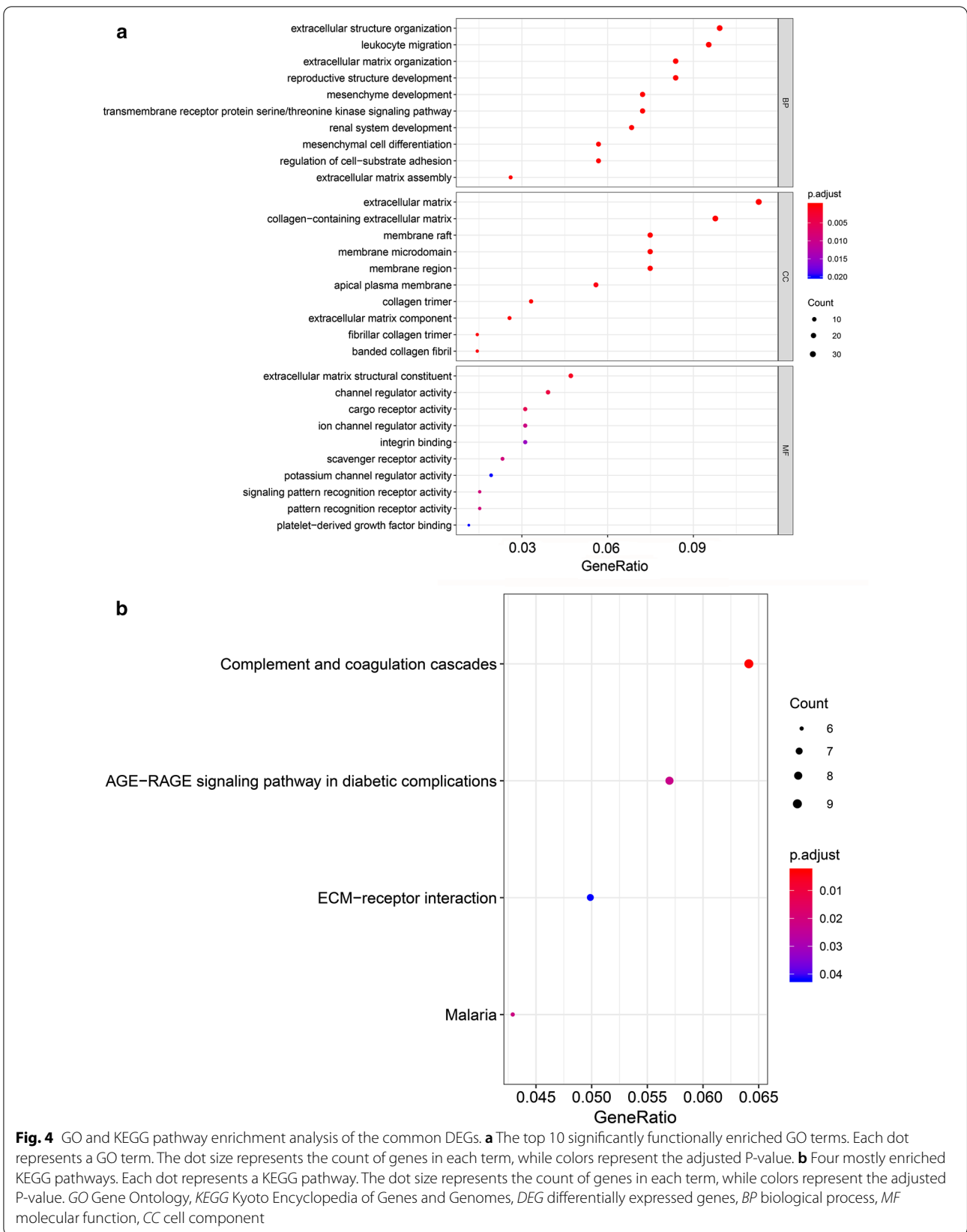
To validate the expression levels of *EZH2* and *HMMR*, expression data from ONCOMINE were compared. The results showed that they were significantly up-regulated in MPM compared to normal tissues (Fig. 6a, b). To investigate the prognostic significance, Kaplan–Meier survival analysis was performed. The log-rank test confirmed that patients with high levels of *EZH2* and *HMMR* had significantly lower survival rate than those with low levels using UALCAN resource (Fig. 7).

### Discussion

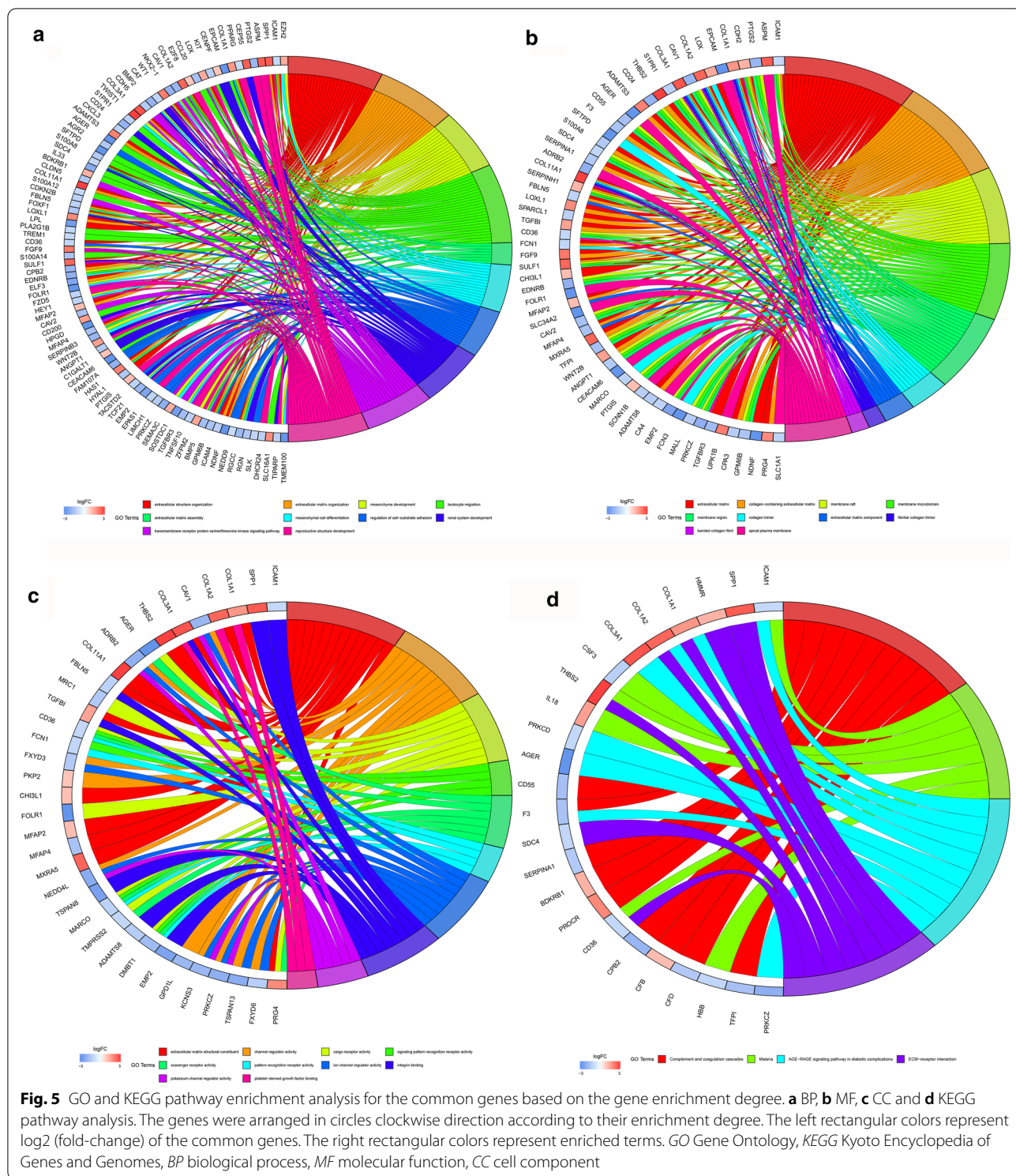
To identify new genes potentially related to MPM, we analyzed two mRNA microarray datasets. A total of 276 common DEGs between MPM tissues and non-cancerous tissues were identified in the 2 datasets, including 187 downregulated genes and 79 upregulated genes. GO and pathway analyses were performed to explore functions of the DEGs. They were found enriched in extracellular structure organization, extracellular matrix, matrix structural constituent, and ECM–receptor interaction. *EZH2*

and *HMMR* were found to be related to MPM based on PPI analysis.

Previous studies have reported that overexpression of HAPLN1, one of the ECM proteins, increases tumorigenic properties of mesothelioma [17]. In addition, high expression of tenascin-C protein, an extracellular matrix glycoprotein known to have anti-adhesive characteristics in MPM, might play a role in invasive growth of MPM [18]. KEGG pathway analysis showed that the common DEGs are associated with complement and coagulation cascades, AGE-RAGE signaling pathway in diabetic complications, ECM–receptor interaction, and *HMMR* was riched in ECM–receptor interaction. Based on parallel sequencing, Hylebos et al. [19] found that tumor protein p53/DNA repair, cell cycle, mitogen-activated protein kinase, and phosphoinositide 3-kinase (PI3K)/AKT pathways are related to MPM. Furthermore, Cedrés et al. proposed that pS6 might be an independent prognostic factor in MPM [9]. Targeting this pathway could be of therapeutic significance. For instance, in the SWOG S0722 trial mTOR inhibitor everolimus (RAD001) has been demonstrated to have limited clinical activity in advanced MPM patients [20].



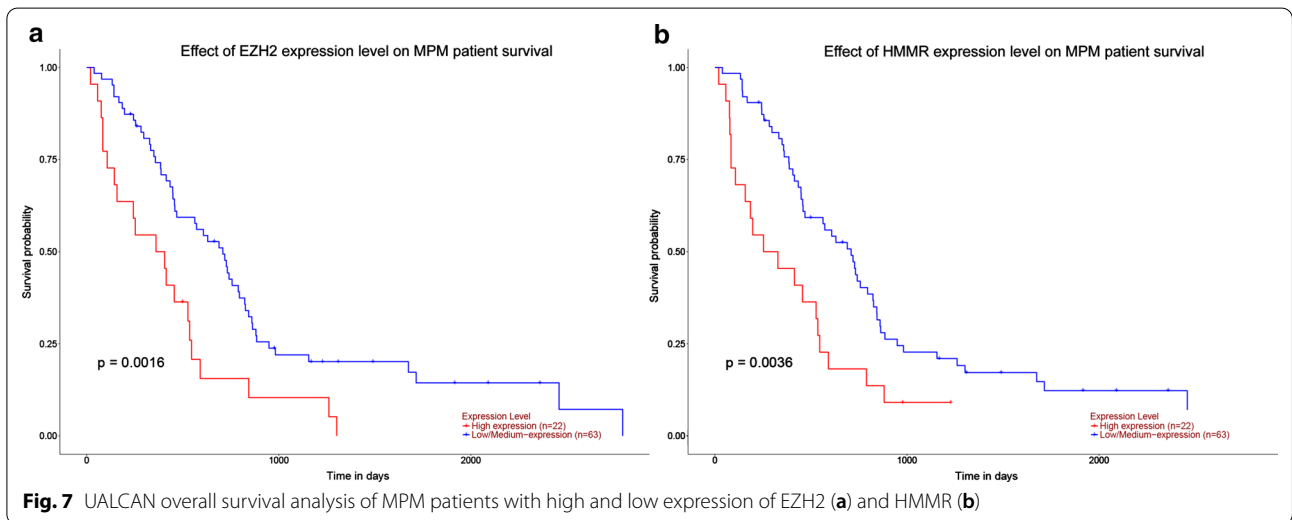
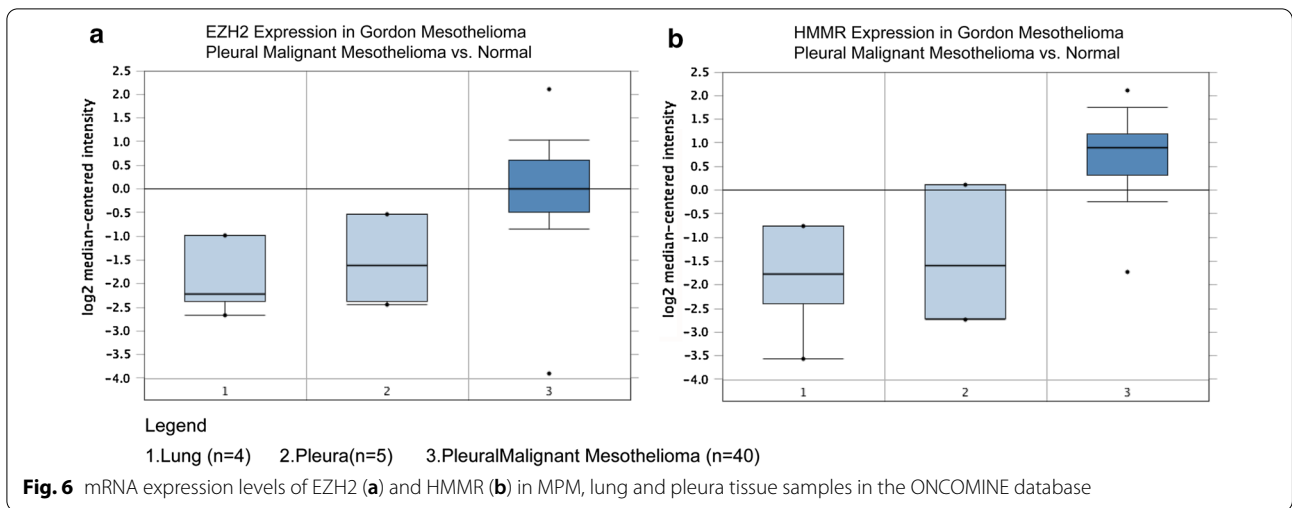
**Fig. 4** GO and KEGG pathway enrichment analysis of the common DEGs. **a** The top 10 significantly functionally enriched GO terms. Each dot represents a GO term. The dot size represents the count of genes in each term, while colors represent the adjusted P-value. **b** Four mostly enriched KEGG pathways. Each dot represents a KEGG pathway. The dot size represents the count of genes in each term, while colors represent the adjusted P-value. *GO* Gene Ontology, *KEGG* Kyoto Encyclopedia of Genes and Genomes, *DEG* differentially expressed genes, *BP* biological process, *MF* molecular function, *CC* cell component



To examine the associations of protein functions of the identified 276 common DEGs, a PPI network was constructed, 24 DEGs were selected as hub genes because of their high degree of connectivity (>19) using MCODE

in Cytoscape. According to the connectivity degree and the node status, EZH2 and HMMR have significant differences between MPM and non-cancerous tissue samples. EZH2 encodes a member of the Polycomb-group





(PcG) family in which the two main families are polycomb repressive complex 1 (PRC1) and PRC2 [21]. They are associated with the embryonic ectoderm development protein, the VAV1 oncoprotein, and the X-linked nuclear protein and may play a role in the hematopoietic and central nervous system [22]. As previously reported, EZH2 is overexpressed in Weaver syndrome and primary cutaneous follicle center lymphoma [22, 23]. LaFave et al. [24] reported that loss of BAP1 promotes MPM cell proliferation by upregulating EZH2. The epigenetic gene H3K27me3 catalyzed by EZH2 is repressive which is aberrantly expressed during malignancy transformation in MPM [25]. Suppression of EZH2 using RNA interference was found to decrease the cancerogenicity of malignant mesothelioma cells [25]. In clinic practice, highly expressed EZH2 and deletion of BAP1 and MTAP

can be detected by immunohistochemistry and may be used to distinguish MPM from mesothelial hyperplasia [21]. HMMR (Hyaluronan Mediated Motility Receptor) is a protein coding gene influencing in cell motility [26]. The protein is detected in breast tissue and complexes with BRCA1 and BRCA2. And thus, HMMR potentially increases the risk of breast cancer. In addition, EZH2 and HMMR were also overexpressed in MPM from ONCOMINE and discovered to be associated with low overall survival in MPM patients, suggesting that they may be potential diagnostic and prognostic biomarkers for MPM.

There are several limitations in this study. The results and conclusions obtained are based on microarray data, and there is a lack of validation with experimental data with clinical samples. The sample size in prognostic analysis is

relatively small. Further biological experiments are needed to elucidate the mechanisms behind the expression changes of these key genes and their biological functions in MPM.

In conclusion, our analysis has identified a number of DEGs that are closely related to the development, progression, and prognosis of MPM. A total of 277 DEGs and 24 hub genes have been found this study. EZH2 and HMMR might be the core genes of MPM based on their high connectivity in the protein interaction network. These findings may provide clues to develop the potential biomarkers for diagnosis of MPM and insight on molecular mechanisms underlying MPM to identify novel pharmacological and therapeutic targets for the treatment of MPM. However, further experimental verification and clinical studies are needed to confirm the potential biological functions and prognostic significance for MPM.

#### Abbreviations

MPM: Malignant pleural mesothelioma; GEO: Gene Expression Omnibus; DEG: Differentially expressed genes; PPI: Protein-protein interaction network; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; SETD2: SET domain containing protein 2; STRING: Search Tool for the Retrieval of Interacting Genes; MCODE: Molecular complex detection; MF: Molecular function; CC: Cellular component; BP: Biological process.

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None.

#### Authors' contributions

XL and YZ designed the study. XL, KQ, GL and PC collected the data and performed analysis. XL, KQ and YZ drafted the manuscript. All authors read and approved the final manuscript.

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#### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

#### Declarations

##### Ethics approval and consent to participate

This work does not involve human or animal study.

##### Consent for publication

Not applicable.

##### Competing interests

The authors declare no competing interests.

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