

Original Article

ANALYSIS OF HKDC1 EXPRESSION IN MULTIPLE MYELOMA: A COMBINED BIOINFORMATIC AND EXPERIMENTAL APPROACH

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ABSTRACT

Hexokinase domain-containing 1 (HKDC1) has been recognised as a crucial protein involved in cancer metabolism. However, its roles in the pathogenesis of multiple myeloma (MM) remain uncertain. This study combined bioinformatic analysis and experimental validation to investigate the expression pattern of HKDC1 in MM. Transcriptomic data from public databases were analysed to explore the expression levels of HKDC1 in different stages of MM. Kaplan-Meier analysis was performed to evaluate the impact of HKDC1 expression on event-free survival (EFS) of MM patients. HKDC1 expression levels were then validated in clinical samples and cell lines using reverse-transcription quantitative polymerase chain reaction (RT-qPCR). Bioinformatic analysis of public databases revealed that HKDC1 was downregulated in monoclonal gammopathy of undetermined significance (MGUS), MM, and relapsed MM compared to the normal plasma cells. Furthermore, low HKDC1 level was found to be associated with shorter EFS in MM patients. Experimental validation via RT-qPCR confirmed that HKDC1 is significantly downregulated in MM patients. Collectively, HKDC1 may play a part in the development and progression of MM, providing new insights into the molecular processes that underlie this condition.

KEYWORDS: HKDC1, multiple myeloma, bioinformatic, transcriptomic, RT-qPCR

INTRODUCTION

Multiple myeloma (MM) is a form of haematologic malignancy characterised by the uncontrolled growth of cancerous plasma cells within the bone marrow (1). The most common clinical manifestations of MM are summarised by the CRAB criteria: hypercalcemia, renal insufficiency, anaemia, and bone fractures. While the therapeutic landscape for MM has advanced significantly in recent years, MM remains largely incurable due to the emergence of resistance to existing therapies (2). Thus, it is critical to develop new therapeutic targets that can provide a more effective response to these patients.

Hexokinase domain-containing 1 (HKDC1) has recently been recognised as a novel fifth hexokinase isozyme in mammals, demonstrating significant involvement in the regulation of glycolysis and lipid metabolism (3). Glycolysis and lipid metabolism are vital to cancer. They control cell growth, invasion, metastasis, and chemoresistance by providing ATP and essential building blocks such as lipids, amino acids, and nucleotides for actively proliferating tumour cells (4). It was demonstrated that metabolic reprogramming via aberrant HKDC1 expression can promote cancer progression and metastasis (5-7). Apart from that, HKDC1 drives cancer progression via mediating cell cycle, DNA replication, and apoptosis mechanisms (8). HKDC1 can also influence tumour microenvironment through immune evasion and metabolic crosstalk (9-10). Aberrant expression of HKDC1 was observed in multiple malignancies, including lung, colorectal, hepatocellular, gastric, and pancreatic cancers (6,9,11-13). According to recent studies, HKDC1 expression is significantly correlated with prognosis in cancer patients (13-16).

Currently, research on the role of HKDC1 in MM pathogenesis is limited. As such, exploring the function of HKDC1 in MM is the goal of this study. Pan-cancer analysis and HKDC1 expression levels in different stages of MM were explored using public databases. The effect of HKDC1 expression on prognosis was predicted using survival analysis. HKDC1 expression level in MM patients and cell lines was then confirmed by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

MATERIALS AND METHODS

Bioinformatic analysis of public databases

Pan-cancer analysis was performed using the UALCAN web portal (17-18). Gene expression microarray profiles GSE125364, GSE5900, and GSE82307 datasets were retrieved from the Gene Expression Omnibus (GEO) database for HKDC1 gene expression analysis (19). The GEO database provides high-quality, independent datasets, which allowed us to validate differential expression gene across different studies, ensuring the robustness and reproducibility of our findings. The selection of the datasets was based on the relevance of sample types, platform consistency, and metadata availability. These datasets consist of samples from the benign stage, monoclonal gammopathy of undetermined significance (MGUS), an asymptomatic intermediate stage, smoldering multiple myeloma (SMM), active stage MM, and the progressive stage or relapsed MM, enabling the investigation of HKDC1 expression in different stages of the disease. GSE125364 contains 3 healthy controls and 45 MM; GSE5900 contains 22 healthy controls, 44 MGUS, and 12 SMM; GSE82307 contains 33 MM and 33 relapsed MM, all derived from the same patients. Event-free survival (EFS) analysis was performed using the GSE24080 dataset (n=559), which is the high-quality and largest MM dataset in the Kaplan-Meier plotter web tool. Patients were divided into low and high HKDC1 based on median expression, and KM analysis was employed to estimate EFS rates as previously described (20).

Clinical samples

A total of 17 samples (13 newly diagnosed MM patients, 2 relapsed MM patients, and 2 healthy controls) were prospectively recruited at the Haematology Department, Ampang Hospital, Kuala Lumpur, Malaysia, between 2009 and 2012. Whole blood or bone marrow aspirates were collected using a consecutive sampling method during routine clinical and diagnostic visits requested by the attending haematologists. For the newly diagnosed MM, inclusion required a confirmed diagnosis of MM with no prior systemic anti-myeloma treatment. In contrast, individuals with concurrent or a history of malignancies or previous treatment were excluded. For the relapsed samples, inclusion required documented disease progression following a clinical response, excluding patients with concurrent or a history of malignancies. Healthy controls included individuals with no clinical evidence of malignancies. The final sample size used for this study was determined by the availability of high-quality archived clinical RNA remnants following prior transcriptomic screening phases.

Cell line and cell culture

KMS-20, KMS-28BM, and KMS-12-BM cell lines were acquired from the cell bank of the Japanese Collection of Research Bioresources (JCRB). RPMI-8226, U266, and MM.1S cell lines were obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured at 37°C in an incubator with 5% CO₂ in RPMI-1640 medium (PAN-Biotech, Germany), supplemented with 10% fetal bovine serum (Sigma-Aldrich, Germany) and 1X antibiotic-antimycotic (Gibco). Cells were passaged every 2-3 days.

Total RNA extraction

The miRNeasy mini kit (Qiagen, Germany) was used to separate total RNAs from the whole blood, bone marrow or cells. DNAs were digested using DNase (Qiagen DNase I, Germany). The Nanodrop ND-1000 UV-VIS spectrophotometer (Thermo Fisher Scientific, USA) was used to measure the purity and concentration of total RNAs.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Applied Biosystems' first-strand cDNA synthesis kit was used to reverse transcribe total RNAs into cDNAs. HKDC1 (Hs00228405_m1, Thermo-Fisher Scientific, USA) and internal control GAPDH (Hs0275899-g1, ThermoFisher Scientific, USA) Taqman gene expression assays were employed in qPCR. The qPCR was carried out in the StepOnePlus™ Real-time PCR System (Applied Biosystems, USA) as follows: initial denaturation at 95°C for 20 s, then 40 cycles of 95°C for 1 s and 60°C for 20 s. The 2^{- $\Delta\Delta C_T$} method was used to determine relative expression.

Statistical analysis

Statistical analysis was performed using R. Mann-Whitney U test (Wilcoxon rank-sum test) was used to find the significant difference between two groups. Statistical significance was defined as a *p*-value of less than 0.05.

RESULTS

HKDC1 expression in cancers

A pan-cancer investigation of HKDC1 mRNA expression revealed that the gene was expressed differently across various types of cancer (Figure 1). Comparable to the normal tissues, HKDC1 expression downregulation was found in thyroid carcinoma (THCA), lung squamous cell carcinoma (LUSC), and cervical squamous cell carcinoma (CESC). Conversely, HKDC1 expression was elevated in colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), liver hepatocellular carcinoma (LIHC), and lung adenocarcinoma (LUAD).

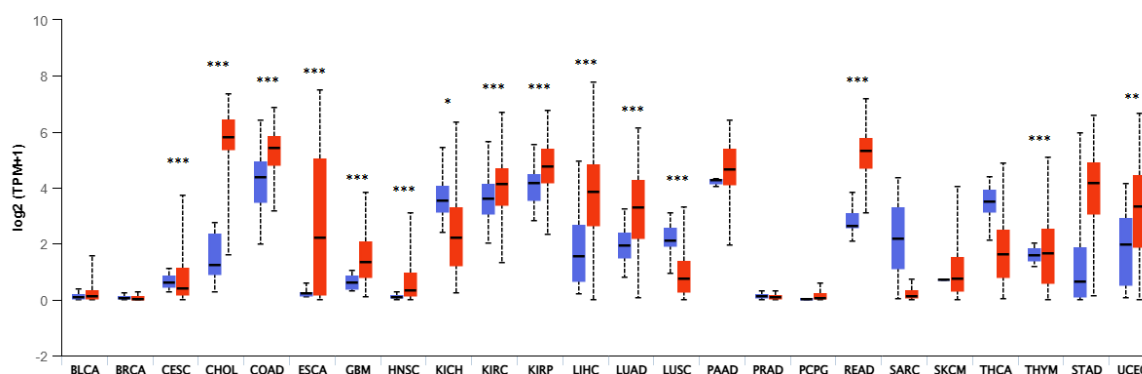


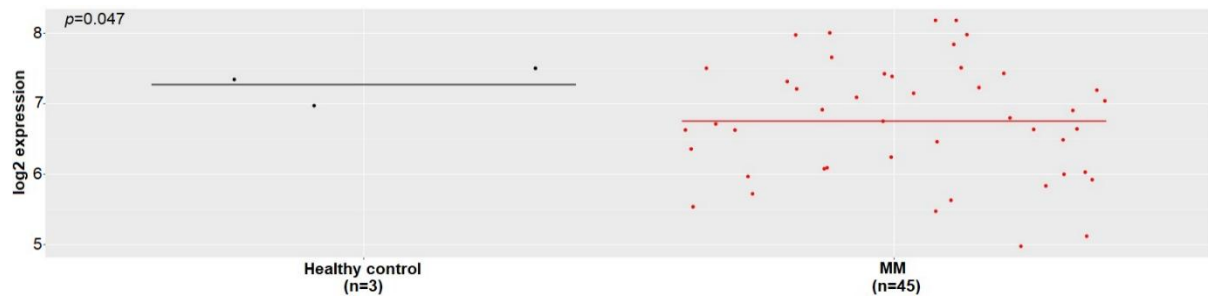
Figure 1. Pan-cancer study of HKDC1 expression. Gene expression of HKDC1 in normal and tumour tissues across cancers. Red represents tumours, while blue represents normal tissues. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma; THYM, thymoma; STAD, stomach adenocarcinoma.

Expression of HKDC1 in different stages of MM

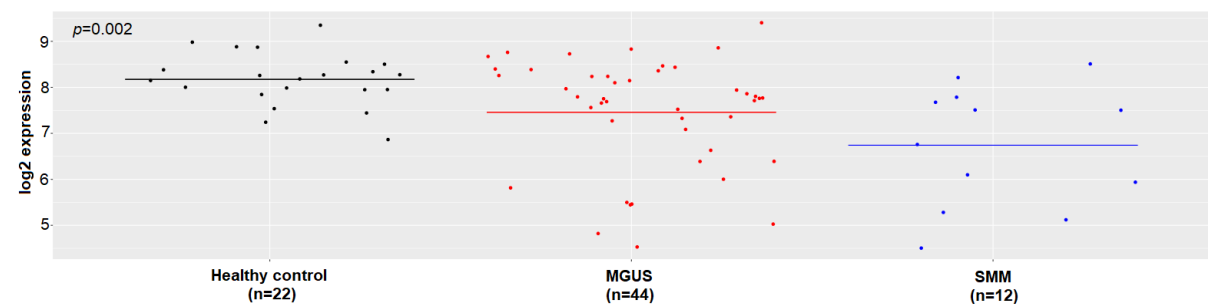
HKDC1 expression in MM was investigated using the GSE125364 dataset. According to the results, the expression level of HKDC1 was lower ($p < 0.05$) in MM compared to normal plasma cells (Figure 2a). The investigation on another dataset, GSE5900, which consists of normal plasma cells, monoclonal gammopathy of undetermined significance (MGUS), and smoldering multiple myeloma (SMM), resulted in HKDC1 expression being lower ($p < 0.01$) in MGUS and SMM compared to the normal plasma cells (Figure 2b). To further explore HKDC1 expression in relapsed MM, the expression of HKDC1 in samples collected at two time points were compared: at initial diagnosis and after relapse or progression following first-line therapy using GSE82307 dataset. Interestingly, HKDC1 expression level was found to be downregulated ($p < 0.05$) in relapsed MM compared to the initial diagnostic samples (Figure 2c).

Association of HKDC1 expression and prognosis in MM

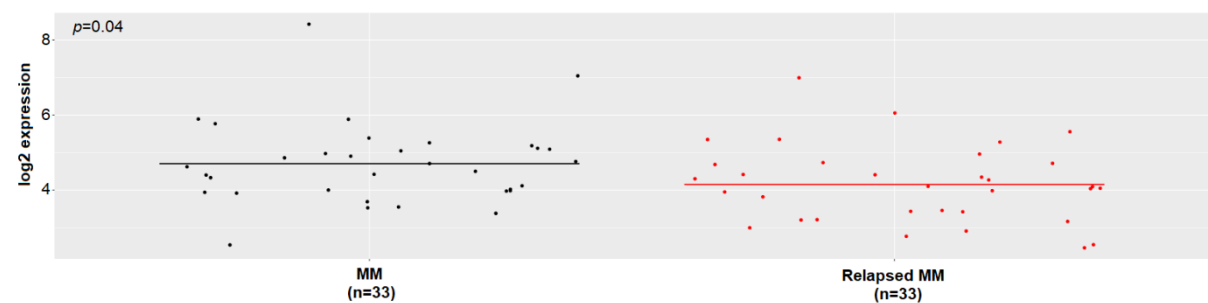
The EFS analysis of the GSE24080 dataset showed that at 10 years of follow-up, EFS was shorter in patients with low expression of HKDC1, suggesting that a reduction of HKDC1 expression may be related to poor prognosis in MM (Figure 2d).



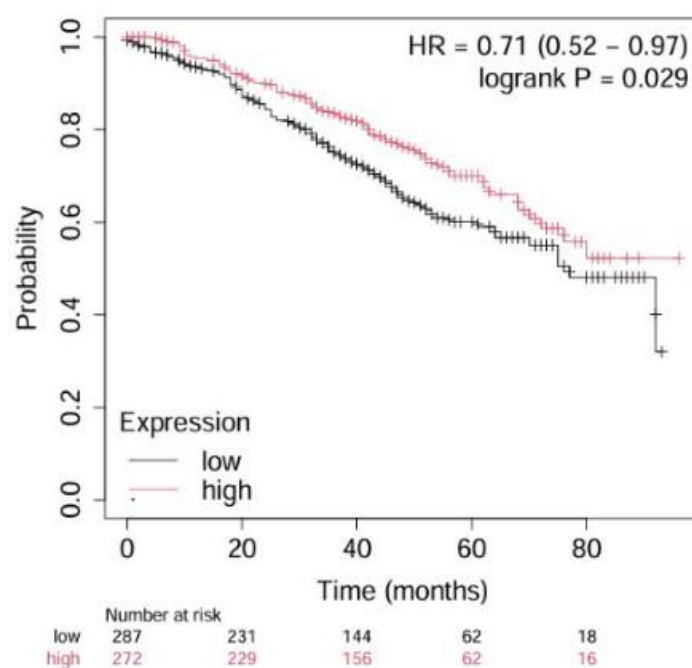
(a)



(b)



(c)



(d)

Figure 2. Bioinformatic analysis of HKDC1 expression in MM. (a) HKDC1 expression was significantly downregulated in MM (n=45) compared to healthy controls (n=3), from the GSE125364 dataset. (b) The expressions of HKDC1 level in MGUS (n=44) and SMM (n=12) were significantly lower compared to healthy controls (n=22), from the GSE5900 dataset. (c) Expression of HKDC1 was significantly downregulated in relapsed MM (n=33) compared to MM from the same patients at initial diagnosis (n=33), from the GSE82307 dataset. (d) EFS analysis showed shorter EFS in MM patients with low HKDC1 expression, from the GSE24080 dataset (n=559). MM, multiple myeloma; MGUS, monoclonal gammopathy of undetermined significance; SMM, smoldering multiple myeloma.

HKDC1 expression in Malaysian MM patients

In this study, HKDC1 expression was validated in clinical samples using RT-qPCR. The results of the RT-qPCR revealed downregulation ($p < 0.05$) of HKDC1 level in MM compared to the healthy controls (Figure 3a). HKDC1 was downregulated in both relapsed MM (12.86-fold, $p = 0.33$) and MM cell lines (12.90-fold, $p = 0.07$) relative to healthy controls, but these changes were not statistically significant (Figure 3a & b).

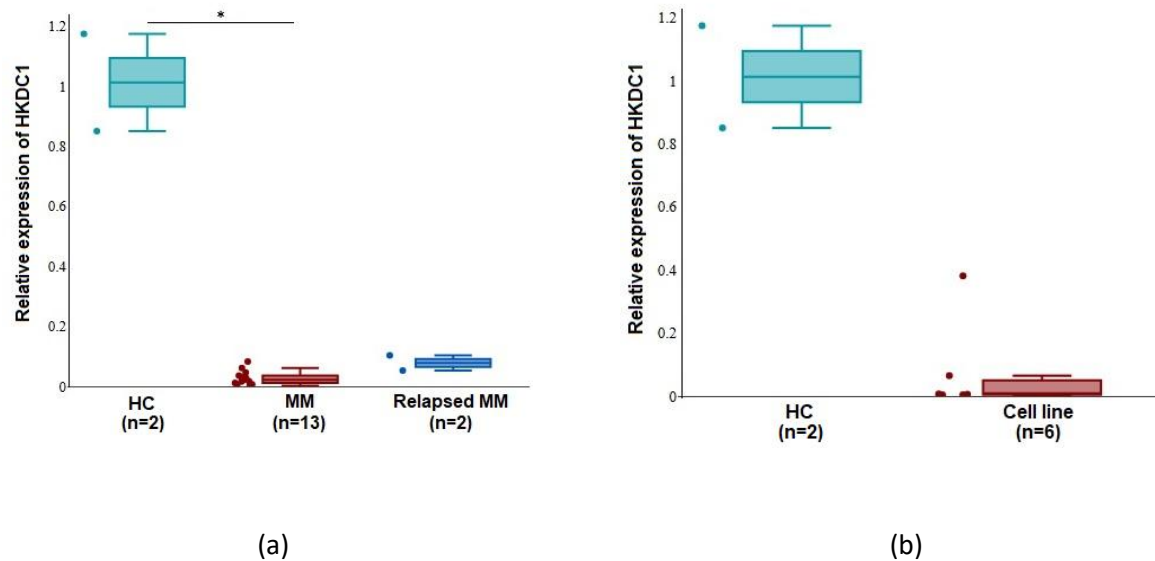


Figure 3. The HKDC1 expression in MM clinical samples and cell lines. (a) HKDC1 expression was significantly downregulated in MM (n=13) compared to the HC (n=2). (b) The expression of HKDC1 was downregulated by 12.90-fold in MM cell lines (n=6) compared to the HC (n=2) ($p=0.07$). * $p<0.05$. HC, healthy control; MM, multiple myeloma.

DISCUSSION

The HKDC1 has been identified as a key protein in cancer metabolism. Aberrant expression of HKDC1 has been shown to correlate with poor clinical outcomes in human malignancies. For the first time, we investigated the roles of HKDC1 in MM pathogenesis using a combined bioinformatic and experimental approach.

According to a pan-cancer analysis, HKDC1 was differentially expressed across cancer types, with expression levels varying among them. Further differential expression analysis of the public database showed significant downregulation of HKDC1 levels in MM relative to normal plasma cells. When HKDC1 expression levels were explored across different stages of MM, it was found that significant downregulation of HKDC1 levels in MGUS (benign precursor to MM) and SMM (asymptomatic MM) compared to healthy donors. Based on this scenario, it appears that decreased HKDC1 expression is a precursory event in the pathophysiology of MM, occurring as early as the benign MGUS stage. Interestingly, analysis of HKDC1 expression levels in paired pre-treatment and relapsed samples revealed a marked reduction in HKDC1 expression in relapsed MM. These findings suggest that HKDC1 may play a role in MM disease progression. To explore the prognostic significance of HKDC1 in MM, an EFS analysis was performed using the GSE24080 dataset. The results demonstrated that shorter EFS was associated with a low level of HKDC1, suggesting a higher risk of relapse, disease progression, or death in patients with low HKDC1 expression. To validate the findings, the relative expression levels of HKDC1 were determined in MM patients (13 newly diagnosed MM, and 2 relapsed MM) and cell lines by RT-qPCR. The RT-qPCR findings revealed that HKDC1 levels were significantly downregulated in MM patients compared to healthy donors, which is in line with the results obtained from the bioinformatic analysis.

HKDC1 is generally upregulated in a wide range of human cancers, including lung, colorectal, hepatocellular, and gastric carcinomas (5-6, 11-13). As HKDC1 is a crucial tumour metabolic regulator, elevated expression of HKDC1 has been shown to act as an oncogene to promote tumour growth, invasion, and chemoresistance via rewiring of glycolysis and oncogenic-related pathways, such as AMPK/mTOR, NF- κ B, Wnt/ β -Catenin and STAT1/PD-L1 signaling pathways (5, 10-11, 21). Elevated HKDC1 expression is associated with poor prognosis in these cancers (21). Conversely, downregulation of HKDC1 is less frequent but has been seen in some cancer types including thyroid carcinoma (THCA), kidney chromophobe (KICH), pancreatic adenocarcinoma (PRAD), and lung squamous cell carcinoma (LUSC). This suggests that the regulation of HKDC1 expression may differ across cancer types (22). DNA copy number loss, genetic variants, and epigenetic modification such as DNA methylation in the HKDC1 promoter region and gene body can result in decreased HKDC1 expression (22). Therefore, the functional mechanisms of HKDC1 are context-dependent, and the role of HKDC1 in MM can be complex, necessitating further investigation.

A limitation of this study is the use of unseparated whole blood or bone marrow aspirates instead of enriched CD138+ plasma cells in RT-qPCR. Although the methodological approach may dilute cell-specific signals, the consistent downregulation of HKDC1 observed in both clinical samples and pure cell lines demonstrates that this suppression is a robust feature of MM. Furthermore, this study is limited by its relatively small sample size, which may have reduced the statistical power of the analyses. Future studies involving larger cohorts are therefore warranted to validate and strengthen the findings of the present study.

CONCLUSION

This study demonstrated that HKDC1 was downregulated in MM, where the decreased expression was significantly associated with MM initiation, progression, and poor prognosis. These findings establish a correlation between the downregulation of HKDC1 expression and MM progression, suggesting its potential as a prognostic biomarker in MM. Further functional assays are necessary to investigate the specific mechanisms of action.

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Conflicts of Interest: Authors have no conflicts of interest to declare.

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