LDHAL6B is a novel prognostic marker and promotes disease progression in multiple myeloma

Multiple myeloma (MM) is an incurable and highly heterogeneous malignancy of plasma cells.[1] To facilitate treatment decision-making, various risk stratification systems have been established to classify MM patients into distinct risk categories at diagnosis. Among these, the International Staging System (ISS) and its successor, the Revised ISS (R-ISS), are widely implemented in clinical practice.[1] However, as treatment regimens for MM continue to evolve, the effectiveness of traditional staging systems and their associated risk factors in predicting patient outcomes may have changed.[2] Furthermore, more than half of MM patients are classified into the R-ISS II category, yet their outcomes can vary considerably.[3,4] To address these challenges, it is essential to identify new risk factors and refine existing staging systems. In this study, we introduced the novel lactate dehydrogenase LDHAL6B as a potential biomarker, which could facilitate the development of risk stratification and personalized treatment strategies for MM.

The "Warburg effect" describes how tumor cells depend on glycolysis for energy, enabling rapid growth even in the presence of oxygen.[5] MM cells, which produce significant quantities of immunoglobulin, are particularly reliant on glycolysis to synthesize amino acid precursors. Lactate dehydrogenase A (LDHA) is crucial to this process, as it catalyzes the conversion of pyruvate to lactate, thereby promoting glycolysis. LDHA plays a significant role in MM by facilitating tumor cell proliferation and drug resistance, and it has been identified as a critical risk factor influencing patient prognosis.[6,7] LDHAL6B, a homolog of LDHA located on chromosome 15 (15q22.2), shares identical structural domains with LDHA. Initially identified in human testes and sperm, LDHAL6B is essential for testicular development and spermatogenesis.[8] While recent studies have focused on its role in regulating glycolysis in animal sperm,[9] its biological and clinical implications in cancer remain largely unexplored.

We initially conducted a bioinformatics analysis of LDHAL6B expression in MM using data obtained from publicly available datasets. The results revealed a significant increase in LDHAL6B expression in MM cell lines (P<0.0001; Figure 1A) and in MM patients (P<0.01; Figure 1B) compared to normal plasma cells. Subsequent validation within our own MM cohort confirmed the upregulation of LDHAL6B at both the mRNA and protein levels in MM patients relative to normal controls (Figure 1C, D). Consistently, MM cell lines also exhibited elevated expression levels of LDHAL6B (*Online Supplementary Figure S1A, B*).

To assess the correlation between LDHAL6B expression and the clinical characteristics of MM patients, bone marrow biopsy samples were collected from 158 MM patients diagnosed at Wuhan Union Hospital between March 2016 and March 2024. Written informed consent was obtained from all participants. This study adhered to the Declaration of Helsinki and received approval from the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology. Immunohistochemical (IHC) staining for CD138 and LDHAL6B was performed on the biopsy samples. The evaluation criteria included staining intensity and the proportion of positively stained areas within the plasma cell regions of the tumor tissue. Staining intensity was categorized as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong), while the proportion of the stained area was classified as 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%). The final score for each specimen was calculated by multiplying the intensity score by the staining area score.[10] A score of ≥3 indicated high LDHAL6B expression, while a score of <3 was classified as low LDHAL6B expression (*Online Supplementary Figure S2A*).

Among the 158 MM cases, 68 patients (43.0%) were classified as the high-expression group. High expression of LDHAL6B was associated with more advanced disease stages, including the ISS (P=0.013), the R-ISS (P<0.001), the Second Revision of the International Staging System (R2-ISS) (P<0.001), and the Mayo Additive Staging System (MASS) (P<0.001) (Table 1). Elevated LDHAL6B expression was also linked to various adverse prognostic factors. Compared to the low-expression group, patients with higher LDHAL6B levels exhibited worse renal function (estimated glomerular filtration rate <60 mL/min/1.73 m², 58.8% *vs*. 37.8%; P=0.009), increased β^2-microglobulin levels (>3.5 mg/L, 89.7% *vs*. 66.7%; P=0.001), elevated serum monoclonal protein concentrations (median 38.7 g/L *vs*. 27.1 g/L; P=0.038), a greater proportion of bone marrow plasma cells (≥30%, 41.2% *vs*. 26.7%; P=0.055), a higher proportion of circulating plasma cells (≥0.038%, 54.4% *vs*. 34.4%; P=0.012), a higher frequency of high-risk cytogenetic abnormalities (HRCA) (47.1% *vs*. 24.4%; P=0.003), and a greater number of bone lesions (≥3, 61.8% *vs*. 41.1%; P=0.012) (Table 1). Furthermore, there was a trend toward reduced expression of CD56 in patients with high LDHAL6B expression (63.2% *vs*. 78.9%; P=0.030). The absence of CD56 is commonly observed in extramedullary disease (EMD) and plasma cell leukemia.[11] Consistently, patients with high LDHAL6B expression were more likely to develop EMD, exhibiting both bone-related (33.8% *vs*. 18.9%; P=0.033) and extraosseous (10.3% *vs*.

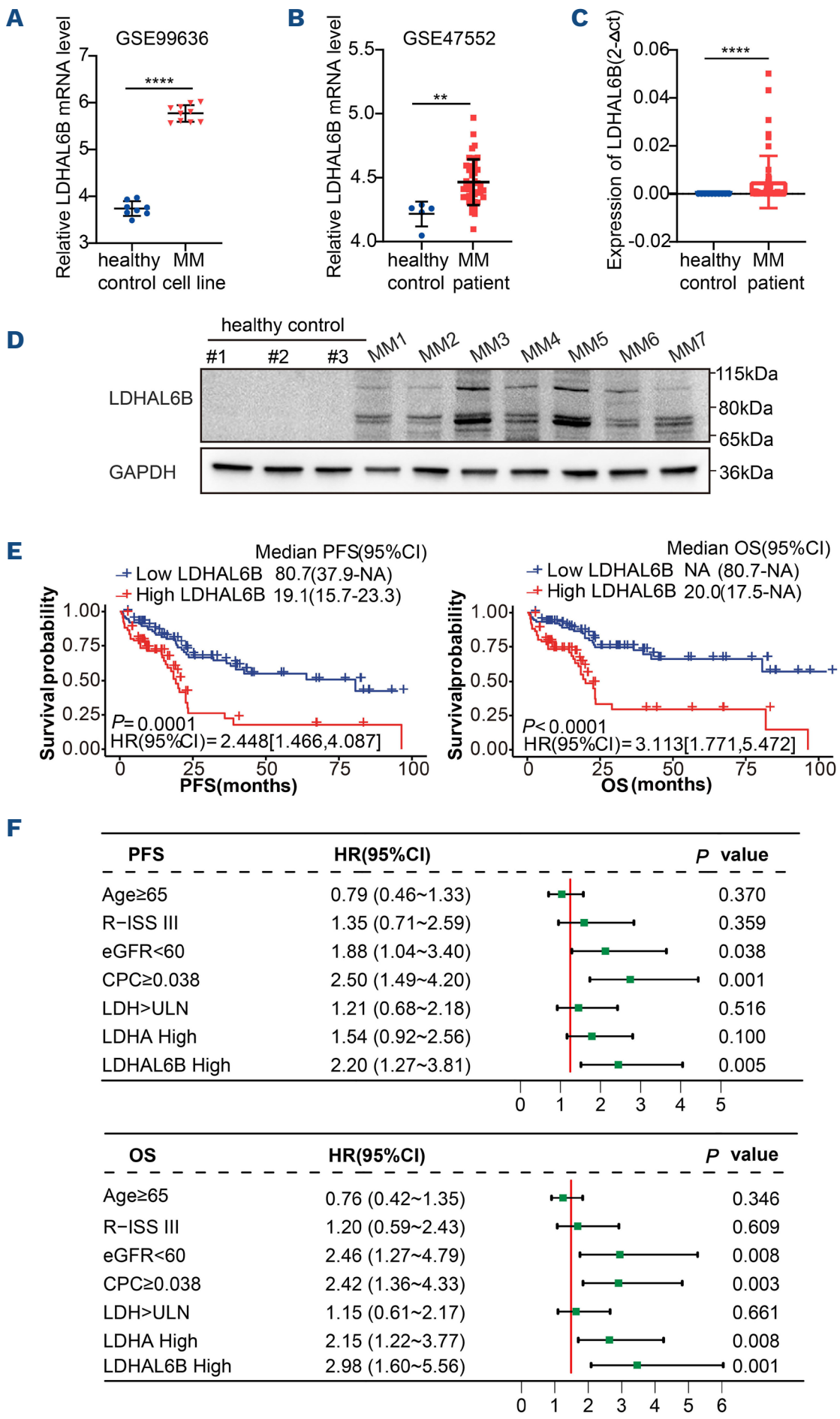

Figure 1. LDHAL6B is upregulated in multiple myeloma and LDHAL6B expression is an independent prognostic factor for multiple myeloma survival. (A) The expression of LDHAL6B in 10 multiple myeloma (MM) cell lines was compared to that in 8 normal plasma cell samples, utilizing the GSE99636 dataset. (B) The expression of *LDHAL6B* in MM patients (N=41) was compared to that in healthy controls (N=5) based on the GSE47552 dataset. (C) The mRNA expression of *LDHAL6B* in MM patients (N=51) and healthy controls (N=10) was quantified using reverse transcription quantitative polymerase chain reaction (RT-qPCR). (D) The protein expression of LDHAL6B in MM patients and normal controls was evaluated through western blot (WB) analysis. (E) Kaplan-Meier survival curves for progression-free survival (PFS) (left panel) and overall survival (OS) (right panel) among MM patients were stratified by high and low LDHAL6B expression levels. *P* values were calculated using the log-rank test. (F) The multivariate Cox regression analysis for PFS (upper panel) and OS (lower panel) of MM patients was conducted. The multivariate analysis was adjusted for age, Revised International Staging System (R-ISS) stage, estimated glomerular filtration rate (eGFR), circulating plasma cells (CPC), serum lactate dehydrogenase (LDH), and LDHA. Healthy control: normal plasma cell; MM1-7: MM patients. The data are presented as the mean ± standard deviation from 3 independent experiments. Statistical significance is indicated as $^{**}P<0.01$ and $^{****}P<0.0001$. HR: hazard ratio; CI: confidence interval; NA: not reached; ULN: upper limit of normal.

Table 1. Correlation between LDHAL6B expression and disease characteristics of multiple myeloma patients.

Characteristic	Total N=158 (100%)	High LDHAL6B N=68 (43.0%)	Low LDHAL6B N=90 (57.0%)	*P*
Sex: male, N (%)	99 (62.7)	44 (64.7)	55 (61.1)	0.644[a]
Age ≥65 years, N (%)	59 (37.3)	30 (44.1)	29 (32.2)	0.126[a]
Isotype: IgH, N (%) IgG IgA IgD Light chain only Other	- 77 (48.7) 30 (19.0) 13 (8.2) 33 (20.9) 5 (3.2)	- 33 (48.5) 13 (19.1) 6 (8.8) 15 (22.1) 1 (1.5)	- 44 (48.9) 17 (18.9) 7 (7.8) 18(20.0) 4(4.4)	0.909[b] - - - - -
Hb <100 g/L, N (%)	103 (65.2)	50 (73.5)	53(58.9)	0.056[a]
Plt <100x10^9/L, N (%)	26 (16.5)	11 (16.2)	15(16.7)	0.934[a]
Alb <35 g/L, N (%)	56 (35.4)	22 (32.4)	34(37.8)	0.480[a]
eGFR[d] <60 mL/min/1.73m^2, N (%)	74 (46.8)	40 (58.8)	34(37.8)	0.009[a]
Ca ≥2.75 mmol/L, N (%)	21 (13.3)	8 (11.8)	13(14.4)	0.623[a]
LDH >ULN, N (%)	39(24.7)	19 (27.9)	20(22.2)	0.409[a]
ISS, N (%) I II III	- 30 (19.0) 41 (25.9) 87 (55.1)	- 5 (7.4) 20 (29.4) 43 (63.2)	- 25 (27.8) 21 (23.3) 44 (48.9)	0.013[c] - - -
R-ISS, N (%) I II III	- 18 (11.4) 88 (55.7) 52 (32.9)	- 3 (4.4) 32 (47.1) 33 (48.5)	- 15 (16.7) 56 (62.2) 19 (21.1)	<0.001[c] - - -
R2-ISS, N (%) I II III IV	- 12 (7.6) 24 (15.2) 80 (50.6) 42 (26.6)	- 0(0.0) 8(11.8) 34(50.0) 26(38.2)	- 12(13.3) 16(17.8) 46(51.1) 16(17.8)	<0.001[c] - - - -
MASS, N (%) I II III	- 26 (16.5) 50 (31.6) 82 (51.9)	- 5 (7.4) 17 (25.0) 46 (67.6)	- 21 (23.3) 33 (36.7) 36 (40.0)	<0.001[c] - - -
β2-MG >3.5 mg/L, N (%)	121 (76.6)	61 (89.7)	60 (66.7)	0.001[a]
BMPC ≥30%, N (%)	52 (32.9)	28 (41.2)	24 (26.7)	0.055[a]
CPC ≥0.038%, N (%)	68(43.0)	37(54.4)	31(34.4)	0.012[a]
Serum M-protein g/L, median (IQR)	32.1(4.8-51.8)	38.7(4.6-63.1)	27.1(4.8-44.3)	0.038[c]
Number of bone lesions, N (%) 0 <3 ≥3	- 62 (39.2) 17 (10.8) 79 (50.0)	- 20 (29.4) 6 (8.8) 42 (61.8)	- 42 (46.7) 11 (12.2) 37 (41.1)	0.012[c] - - -
EMD-B, N (%)	40 (25.3)	23 (33.8)	17 (18.9)	0.033[a]
EMD-E, N (%)	8 (5.1)	7 (10.3)	1 (1.1)	0.021[b]
CD56, N (%)	114 (72.2)	43 (63.2)	71 (78.9)	0.030[a]
HRCA, N (%) Any HRCA t (4;14) t (14;16) del(17p)	- 54 (34.2) 35 (22.2) 13 (8.2) 23 (14.6)	- 32 (47.1) 21 (30.9) 10 (14.7) 12 (17.6)	- 22 (24.4) 14(15.6) 3 (3.3) 11 (12.2)	- 0.003[a] 0.022[a] 0.010[a] 0.338[a]
≥VGPR, N (%)	71 (44.9)	23 (33.8)	48 (53.3)	0.019[a]
MRD-, N (%)	28 (17.7)	7 (10.3)	21 (23.3)	0.034[a]

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[a]*P* value was calculated by χ^2 test. [b]*P* value was calculated by Fisher's exact test. [c]*P* value was calculated by Mann-Whitney U test. [d]eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. N: number; MM: multiple myeloma; ISS: International Staging System; R-ISS: Revised International Staging System; R2-ISS: second revision of the International Staging System; MASS: Mayo Additive Staging System; Hb: hemoglobin; Plt: platelet; Alb: albumin; eGFR: estimated glomerular filtration rate; Ca: calcium; LDH: lactate dehydrogenase; ULN: upper limit of normal; β2-MG: β2-microglobulin; CPC: circulating plasma cell; BMPC: bone marrow plasma cell; IQR: interquartile range; EMD-B: bone-related extramedullary disease; EMD-E: extraosseous extramedullary disease; HRCA: high-risk chromosome abnormality; del: deletion; t: translocation; VGPR: very good partial response; MRD-: minimal residual disease negativity.

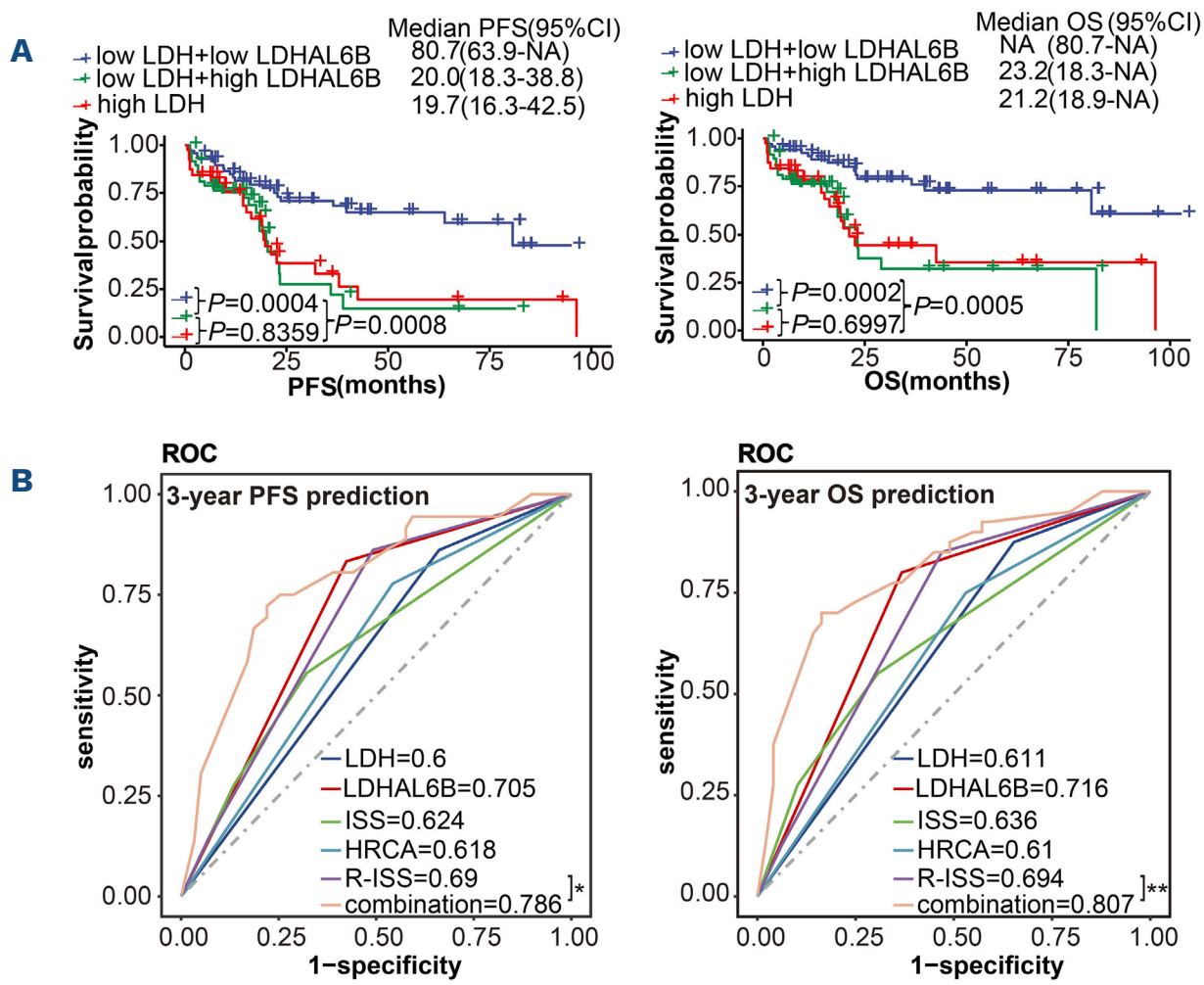
1.1%; *P*=0.021) manifestations (Table 1). These findings suggest that LDHAL6B may confer more aggressive biological characteristics to MM cells.

The response to first-line therapy is crucial in determining patient outcomes.[12] Our research indicated that patients with high LDHAL6B expression exhibited a lower rate of achieving a very good partial response (33.8% *vs*. 53.3%; *P*=0.019) and were less likely to attain minimal residual disease negativity compared to those with low expression levels (10.3% *vs*. 23.3%; *P*=0.034) (Table 1).

To evaluate the consistency among tissue LDHAL6B expression, LDHA expression, and serum LDH levels, we conducted a parallel assessment of LDHA expression in bone marrow biopsy samples from 158 MM patients, utilizing the same IHC scoring criteria applied to LDHAL6B. A score of ≥8 indicated high LDHA expression, while a score of <8 indicated low LDHA expression. The K test demonstrated that the expression levels of tissue LDHAL6B, LDHA, and serum LDH were not fully consistent (*Online Supplementary Figure S2B-D*). Further analysis revealed that patients with low LDHAL6B expression in tissues also exhibited low serum LDH levels. Conversely, only a minority of patients with high LDHAL6B expression in tissues showed elevated serum LDH levels (*Online Supplementary Figure S2C*). Similarly, LDHA expression in tissues demonstrated a comparable correlation with serum LDH levels (*Online Supplementary Figure S2D*).

Subsequently, we investigated the correlation between LDHAL6B expression and patient survival. The median follow-up duration was 17.7 months. Patients with high LDHAL6B expression had inferior progression-free survival (PFS) (median 19.1 *vs*. 80.7 months; *P*=0.0001) and overall survival (OS) (median 20.0 *vs*. not reached; *P*<0.0001) compared to those with low LDHAL6B expression (Figure 1E). Multivariate analysis confirmed LDHAL6B as an independent adverse prognostic factor for PFS (hazard ratio [HR] =2.20; 95% confidence interval [CI]: 1.27-3.81; *P*=0.005) and OS (HR=2.98; 95% CI: 1.60-5.56; *P*=0.001) (Figure 1F).

Elevated serum levels of LDH are associated with in-



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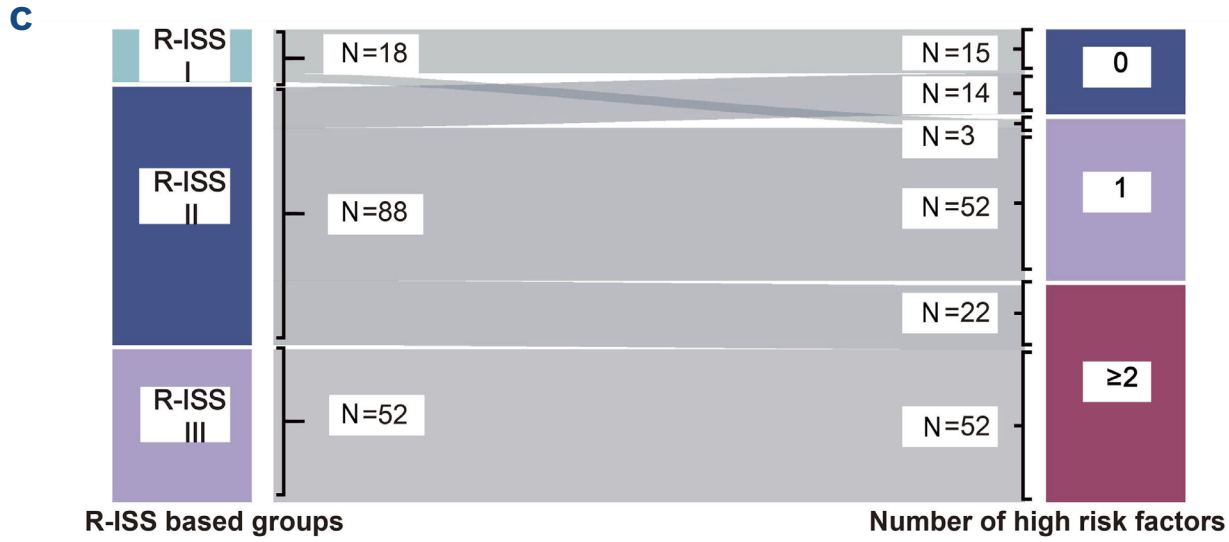

Figure 2. LDHAL6B overexpression significantly enhances prognostic utility of clinically established multiple myeloma markers. (A) Kaplan-Meier survival curves for progression-free survival (PFS) (left panel) and overall survival (OS) (right panel) of multiple myeloma (MM) patients based on LDHAL6B expression combined with serum lactate dehydrogenase (LDH) levels. *P* values were calculated using the log-rank test. (B) Receiver operating characteristic (ROC) curves were generated to predict 3-year PFS (left panel) and OS (right panel) based on the Revised International Staging System (R-ISS), LDHAL6B expression, and their combination. The area under the curve (AUC) for PFS was 0.690 for R-ISS, 0.705 for LDHAL6B, and 0.786 for their combination. The AUC for OS was 0.694 for R-ISS, 0.716 for LDHAL6B, and 0.807 for their combination. (C) Alluvial diagram illustrating the transition from R-ISS categories to a classification based on the number of risk factors. (D) PFS (left panel) and OS (right panel) according to the R-ISS. (E) PFS (left panel) and OS (right panel) based on the number of risk factors, which include high LDHAL6B expression, International Staging System (ISS) stage III, elevated serum LDH, and high-risk chromosome abnormality (HRCA) (del(17p), t(4;14), t(14;16)). **P*<0.05; ***P*<0.01. N: number; del: deletion; t: translocation.

creased disease aggressiveness and a higher proliferation rate of MM cells.[13] Due to their significant impact on patient survival, elevated LDH levels have been incorporated into the R-ISS and various other clinical risk stratification models.[14] Here, we found that 75.3% (119/158) of patients exhibited low serum LDH levels. Among these patients, those with elevated LDHAL6B expression experienced poorer PFS and OS, similar to patients with high serum LDH levels (Figure 2A). The inclusion of LDHA expression did not effectively stratify patients with low serum LDH levels (*data not shown*), suggesting that LDHAL6B, rather than LDHA, may more accurately address the limitations of serum LDH in the prognostic assessment of MM patients.

To further refine prognostication, we developed a novel model integrating high LDHAL6B expression with high-risk factors from the R-ISS, which includes ISS stage III, elevated serum LDH, and HRCA (del(17p), t(4;14), t(14;16)). Receiver operating characteristic curve analysis demonstrated that this combined model significantly enhanced

the prediction of 3-year PFS and OS compared to the R-ISS alone (Figure 2B). In this new model, patients were classified into three risk categories: low-risk (no high-risk factor), intermediate-risk (1 high-risk factor), and high-risk (2 or more high-risk factors) (Figure 2C). Compared to the R-ISS (Figure 2D), the new prognostic model demonstrated superior patient stratification for prognosis (Figure 2E). This improvement may be attributed to the complementary role of LDHAL6B in relation to serum LDH.

To investigate the role of LDHAL6B in MM, we established MM cell lines with stable knockdown and overexpression of *LDHAL6B* (*Online Supplementary Figure S3A*). Functionally, the knockdown of *LDHAL6B* significantly inhibited cell proliferation, migration, and invasion, whereas overexpression enhanced these processes (*Online Supplementary Figure S3B, C*). Moreover, LDHAL6B could influence the production of intracellular lactate in MM cells (*Online Supplementary Figure S3D*). Notably, protein-protein interaction analysis indicated that LDHAL6B interacts with enzymes crucial for glucose metabolism, amino acid metabolism, and the tricarboxylic acid cycle (*Online Supplementary Figure S3E*). The Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses of proteins interacting with LDHAL6B demonstrated significant enrichment in critical energy-related pathways (*Online Supplementary Figure S3F*). These findings suggest that LDHAL6B is involved in the metabolic reprogramming of MM cells, a process likely to enhance tumorigenicity and invasiveness.[15]

The present study constitutes the inaugural report on the dysregulation of LDHAL6B in MM and highlights its potential utility in enhancing patient risk stratification and prognostic evaluation.

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Disclosures

No conflicts of interest to disclose.

Contributions

LZ and CS conceived and designed the study. FF, BZ, FZ, SL, and YH provided study materials or patients. LZ, ZL, LC, and QL conducted experiments and performed data analysis. LZ, ZL, LC, and CS discussed and interpreted the data. FF and YZ provided guidance on the content and grammar of the manuscript. LZ and CS wrote the manuscript, and all authors reviewed and approved it.

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Data-sharing statement

Public datasets related to this article are available at the GEO under accession numbers GSE99636 and GSE47552 (https://www.ncbi.nlm.nih.gov/geo/). Additional data can be requested from the corresponding author upon reasonable request.

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