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Exploring secondary extramedullary myeloma disease: a five-predictor scoring system with spotlight on double-hit cytogenetics

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Abstract

Background Extramedullary myeloma disease (EMD) can present at disease relapse (secondary EMD, sEMD) and confers an aggressive clinical course. Identifying predictive markers for sEMD is crucial for clinical management.

Methods Our study, spanning February 2013 to October 2022, identified sEMD in 77 (12.5%) of 618 newly diagnosed multiple myeloma patients. We categorized sEMD patients into bone-related extramedullary (EM-B) and extraosseous extramedullary (EM-E) relapse groups, as well as into early and late relapse groups based on the median interval from initial MM diagnosis, and assessed their overall survival (OS). We investigated independent predictors for the development of sEMD and focused on double-hit (DH) myeloma, one of the predictors of sEMD. Through the analysis of single-cell RNA from DH myeloma samples, we explored the potential mechanisms by which it may contribute to sEMD.

Results Median OS post-sEMD diagnosis was 11 months, with no significant OS difference between EM-B and EM-E relapse groups. A median interval of 22 months from initial MM diagnosis to sEMD relapse divided the 77 sEMD patients into early and late relapse groups, with early sEMD associated with significantly inferior OS post-sEMD (5.0 vs 27.0 months, $p = 0.028$). Driven by the prognostic difference of early vs late sEMD relapse, we used a time-to-event model and identified five independent predictors: double-hit (DH) cytogenetics, ≥ 3 osteolytic lesions, IgD subtype, and non-autologous stem-cell transplantation (ASCT) status, each scoring one point, alongside EM-E scoring two points. These predictors informed an additive score, stratifying patients into low (0–2 points) and high (3–5 points) risk categories for sEMD, showing a significant difference in 3-year sEMD rates (6.6% vs 52.8%, $p < 0.001$). Moreover, the single-cell RNA sequencing of newly diagnosed DH myeloma samples uncovered significant mitogen-activated protein kinase (MAPK) activation in DH cells and exhaustion in CD8 + memory and NK effector cells. Potential therapeutic targets such as EZH2 have emerged from this analysis.

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Conclusions Our study introduces a five-predictor scoring system informed by the potential mechanisms underlying sEMD progression in DH myeloma, with the goal of delaying or possibly preventing sEMD.

Keywords Secondary extramedullary myeloma, Double-hit myeloma, Predictor, Overall survival, Single-cell RNA sequencing

Background

Multiple myeloma (MM), a malignancy characterized by the proliferation of aberrant plasma cells closely interacting with the bone marrow milieu, has seen considerable advancements in overall survival (OS) over the last 15 years [1]. However, MM remains predominantly incurable, with most patients experiencing eventual relapse and developing treatment-resistant disease. Secondary extramedullary myeloma disease (sEMD), manifesting at relapse, is associated with a more aggressive disease course [2] and variable reported incidence rates, ranging from 7.5 to 30% [3–5]. Median survival following sEMD onset is critically low, approximately 6–9 months [2, 6]. Current standard regimens for relapsed/refractory MM (RRMM), including agents like bortezomib, lenalidomide, and pomalidomide, fail to considerably enhance sEMD outcomes [7, 8]. The introduction of anti-CD38 antibodies, such as daratumumab and isatuximab, has not translated into improved prognoses, potentially due to low expression of the CD38 on EMD plasma cells [9]. Novel therapies like chimeric antigen receptor T-cell (CART) treatments and bispecific T-cell engagers show reduced efficacy and survival benefits in sEMD patient populations [10, 11], despite their notable success in treating triple-class refractory myeloma. Even among patients who initially respond to treatment, the therapeutic benefits are often short-lived [2]. Consequently, identifying predictive markers for sEMD is crucial for clinical management. However, the absence of large-scale prospective trials has resulted in insufficient clinical data to define the predictive traits. Thus, our study examines the prevalence, clinical attributes, and survival outcomes of sEMD, alongside risk factors for its development, in a cohort of 618 patients with newly diagnosed MM (NDMM). Additionally, we utilize single-cell sequencing (scRNA-seq) analysis to further explore the potential mechanisms underlying the progression of sEMD in double-hit (DH) myeloma. This real-world analysis of sEMD risk factors and mechanistic exploration of DH samples aim to inform the identification and prophylaxis of sEMD amidst emerging therapeutics.

Methods

Patient cohort selection and cytogenetic risk assessment

Following ethical committee approval, we included NDMM patients presenting with symptomatic and quantifiable disease as per the International Myeloma Working Group (IMWG) guidelines [12]. Individuals with solitary plasmacytomas or initial primary plasma cell leukemia were excluded from the study. Patients with MM and concurrent AL amyloidosis were included in the study and comprised 2.5% (15/611) of the total cohort based on available data. Our retrospective analysis incorporated 618 NDMM patient records from Ruijin Hospital between February 2013 and October 2022, with a follow-up endpoint of November 30th, 2023. Cytogenetic risk was centrally assessed by fluorescence in-situ hybridization (FISH) analysis on CD138-positive sorted cells (200 nuclei analyzed) from bone marrow samples at diagnosis. For patients with concurrent ≥ 2 cytogenetic abnormalities deemed high-risk, including t(4;14), t(14;16), 1q21 gain/amplification (1q21 +), and del(17p), known as "double hit" myeloma (DHMM) [13]. The established thresholds for cytogenetic abnormalities were $\geq 10\%$ for translocations and $\geq 20\%$ for copy number variations [14].

Treatment protocol

The treatment protocol consisted of induction, autologous stem-cell transplantation (ASCT) for eligible patients, consolidation therapy as indicated, and maintenance. Induction involved 4–8 cycles of 28 days with the PAD regimen: bortezomib (1.3 mg/m^2 on days 1, 4, 8, 11) and pegylated liposomal doxorubicin (30 mg/m^2 over 3 days in cycle 1), plus dexamethasone (40 mg on days 1–4). Induction was augmented with lenalidomide (10 mg on days 1–21) and/or daratumumab (16 mg/kg on days 1, 8, 15) during cycles 3–4 for patients demonstrating suboptimal response (< partial response) or possession of del(17p), or t(4;14), or t(14;16) after two cycles. Post-induction, ASCT candidates underwent stem cell collection and ASCT after 4 cycles of induction. After subsequent response assessment (within 100 days post-ASCT), those achieving less than a complete response received a further four cycles of consolidation therapy, as previously described [15]. Patients ineligible for ASCT continued with their original induction therapy for a total

of eight cycles. Maintenance therapy across all patient groups involved lenalidomide (10 mg on days 1–21 of a 28-day cycle) and/or bortezomib (1.3 mg/m^2 biweekly) for up to 2 years.

Outcome measures and diagnostic assessments

Progression-free survival (PFS) was defined as the time from the initialization of therapy to progression according to IMWG criteria [12] or death by any cause. OS was defined as the time from the initialization of therapy to death by any cause. Patients who did not die or were lost to follow-up were censored at their last evaluation of myeloma status. Prior to initial treatment and at the time of sEMD relapse, all participants underwent diagnostic imaging, including positron emission tomography-computed tomography, computed tomography, B-ultrasound, or local magnetic resonance imaging. This study included baseline PET/CT for 72.3% (447/618) patients and other PET/CT at relapse for 74.0% (57/77) patients. Among 362 patients with ≥ 3 osteolytic lesions, 86.2% ($n=312$) had PET/CT, 5.8% ($n=21$) had CT, 7.2% ($n=26$) had X-ray, and 0.8% ($n=3$) had MRI. EMD presentations were verified by biopsy and histological examination whenever clinically indicated and safely executable. Bone-related extramedullary (EM-B) disease was defined as malignant plasma cells breaking through the cortical bone but only forming a soft tissue mass around the bone; extraosseous extramedullary (EM-E) disease referred to malignant plasma cells forming soft tissue masses distal to bone structures [16, 17]. EMD were classified into EM-B or EM-E categories, with the EM-E category encompassing any EM-E manifestations, irrespective of concurrent EM-B presence.

Characteristics of single-cell RNA-seq data

We re-analyzed the datasets of single cell from human bone marrow mononuclear cells from eight normal individuals (data from the R package SeuratData::hcabm40k) and five newly diagnosed DHMM patients. Specifically, GSM3272433, GSM3272434, GSM3272435, and GSM3272436 were defined as MM01 with 1q + and t(14;16) [18]; GSM5702272 was defined as MM163 with 1q + and t(4;14); GSM5702276 was defined as MM203 with 1q + and del(17p); GSM5702278 was defined as MM220 with 1q + and t(4;14) [19]; GSM3528764 was defined as R9 with 1q + and t(4;14) [20] in this study.

Single-cell RNA-seq data integration and analysis

All the matrix was merged and the batch effect was removed with the R package harmony v1.2.0. Following the common pipeline of the R package Seurat 4.4.0, the merged expression matrix was normalized by NormalizeData function and the top 3000 highly variable genes were calculated by Seurat:: FindVariableGenes function. The uniform manifold approximation and projection (UMAP) dimensionality reduction was used to project these populations in two dimensions. The Seurat::FindAllMarkers was also performed to select signature genes in different cell clusters. The function FeaturePlot and VlnPlot in R package Seurat (v4.4.0) and the function DotPlot in the R package ggplot2 (v3.4.4) were used to visualize the gene expression.

Gene set enrichment analysis (GSEA) and visualization

The function gseGO and gseKEGG in the R package clusterProfiler (v4.4.4) were used to implement the GSEA enrichment. The R package enrichplot (v1.16.2) was used to visualize the enrichment results obtained from GSEA analysis.

Cell–cell communication and ligand-receptor interaction analysis

The R package cellchat (v2.1.1) was applied to investigate the cell–cell communication; ligand–receptor interaction with P-value < 0.05 was considered as significant. The expressions of ligands and receptors were visualized with the R package ggplot2 (v3.4.4).

Statistical methods

Continuous variables were presented with medium plus range and were compared using nonparametric tests. Categorical data were expressed with proportions and were evaluated through the chi-square or Fisher's exact test as appropriate. Time-to-event outcomes were calculated with the Kaplan–Meier method and compared using the log-rank test. Follow-up duration was determined via reverse censoring [21]. The univariate Cox proportional hazards model identified potential risk factors for sEMD, and a multivariate Cox model identified independent predictors, incorporating only variables significant ($p<0.05$) in univariate analysis. The point estimate for hazard ratio (HR) together with its 95% confidence interval (CI) was calculated. Predictors were assigned scores based on their Cox model coefficients relative to that of extensive bone lesions (the median coefficient used as a reference, score value = 1) [22, 23]. These scores were then rounded to the nearest integer. Cumulative incidence curves for sEMD were stratified by additive scores and risk groups. Risk stratification based on the scoring system categorized patients as low risk (score 0–2) and high risk (score 3–5). Statistical analyses were performed using SPSS (version 22.0) and the R packages survival and survminer (R/Bioconductor version 4.3.1), with a two-sided significance threshold of $p<0.05$.

Results

Clinical characteristics and involved sites of sEMD

Between February 2013 and October 2022, with a median follow-up of 39 months, 77 patients (12.5%) developed sEMD among 618 NDMM patients. The demographic and pathological characteristics as well as clinical features at the time of MM diagnosis were compared between the sEMD and non-sEMD groups (Table 1), revealing correlations with certain clinical, laboratory, and cytogenetic markers. Briefly, patients with NDMM progressing to sEMD presented with a higher frequency of ≥ 3 osteolytic lesions (83.1% vs 55.1%; $p < 0.001$), hypercalcemia (13.0% vs 5.0%; $p = 0.006$), and EM-E at diagnosis (15.6% vs 3.0%; $p < 0.001$) compared to those without sEMD. The sEMD cohort had a lower rate of ASCT (24.7% vs 39.9%; $p < 0.001$). Cytogenetically, an increased incidence of gain/amplification at the 1q21 locus (65.6% vs 51.7%; $p = 0.038$) and double-hit (DH) cytogenetic abnormalities (34.5% vs 14.6%; $p < 0.001$) were observed in the sEMD group, indicative of a more aggressive disease course. No significant differences were found in age (≤ 65 years), gender, ISS or r-ISS stage, renal dysfunction, anemia, elevated LDH levels, monoclonal protein (M protein) type, induction therapy, or the existence of EM-B at diagnosis between the two groups.

In characterizing sEMD, our study included both secondary EM-B (sEM-B) and secondary EM-E (sEM-E) types of plasmacytomas. Of the patients with sEMD, involvement of sEM-B was documented in 40.3% ($n = 31$). The most prevalent sEM-B locations were paravertebral regions (35.5%), followed by the chest (29.0%), skull (19.4%), pelvis (9.7%), and shoulder blade or long bones (each 6.5%). As for sEM-E, the most commonly affected sites included soft tissue/skin/muscle (39.1%), pleural and lung tissues (21.7%), central nervous system (15.2%), and lymph nodes (13.0%). Less common sites comprised liver (4.3%) and an aggregate of breast, kidney, and pancreas involvement (6.5%) (Additional file 1: Table S1).

Survival outcomes and sEMD relapse patterns in newly diagnosed and relapsed MM patients

In a cohort of 618 NDMM patients, 325 experienced disease relapses, with 77 (36.4%) cases evolving to sEMD, which encompasses both sEM-B and sEM-E lesions. At the end of the follow-up period, the mortality rate was 21%, corresponding to 130 patients deceased, and the estimated median OS for the cohort was 7.7 years (Fig. 1A). Notably, the subgroup with sEMD relapse demonstrated considerably inferior outcomes, with an estimated median OS of 3.7 years (95% CI: 2.7–4.7 years) from the initial diagnosis of MM (Fig. 1A). Survival analysis indicated that the median OS post-relapse was significantly lower for patients with sEMD compared to those relapsed but without sEMD (11.0 months vs 73.0 months, $p < 0.001$, Fig. 1B). After a median follow-up of 27 months post-sEMD diagnosis (range: 1–55 months), OS between sEM-B and sEM-E groups was not statistically different despite a trend towards shorter survival in the sEM-E (18.0 months vs 9.0 months, $p = 0.210$, Fig. 1C).

A median duration of 22 months (Fig. 1D) was observed between initial MM diagnosis and sEMD relapse among 77 patients. These patients were stratified into early (≤ 22 months) and late (> 22 months) sEMD cohorts. Early sEMD relapse was associated with a significantly decreased OS following sEMD (5.0 months vs 27.0 months, $p = 0.028$, Fig. 1E), as well as a significantly worse OS from the initial MM diagnosis (23.0 months vs 65.0 months, $p < 0.001$, Fig. 1F), compared to those with late sEMD relapse.

DH cytogenetics as a significant predictor of sEMD relapse in time-dependent univariate and multivariate analyses

The prognostic implications of early vs late sEMD relapse indicate that timing should be considered in risk stratification for sEMD. Univariate Cox regression analysis identified DH cytogenetics, along with ≥ 3 osteolytic lesions, EMD at diagnosis (encompassing EM-E and EM-B subtypes), hypercalcemia, IgD subtype, absence of ASCT (non-ASCT), increased LDH, R-ISS stage III, and gain/amplification of 1q21 as significant predictors of sEMD relapse (each with $p < 0.05$). ISS stage III ($p = 0.070$), del(17p) ($p = 0.073$), and t(4;14) ($p = 0.092$) demonstrated a trend toward an association, whereas factors such as age at MM diagnosis (age ≤ 65 years), sex, induction regimen, other myeloma protein types, renal impairment, anemia, and t(11;14) did not significantly influence sEMD relapse in the univariate analysis (Fig. 2A). The subsequent multivariate analysis, which controlled for significant variables from the univariate stage, indicated that five covariates retained their independent prognostic value for predicting sEMD relapse: DH cytogenetics (HR 2.20, 95% CI, 1.16–4.21, $p = 0.017$), EM-E at diagnosis (HR 6.12, 95% CI, 2.69–13.92, $p < 0.001$), presence of ≥ 3 osteolytic lesions (HR 3.15, 95% CI, 1.45–6.82, $p = 0.004$), IgD MM subtype (HR 3.48, 95% CI, 1.42–8.53, $p = 0.007$), and non-ASCT status (HR 2.10, 95% CI, 1.14–3.86, $p = 0.017$) (Fig. 2B).

Development of the sEMD predictive scoring system in NDMM patients

The five predictors significantly affecting sEMD relapse were used to build an additive score. In the scoring system, as detailed in the Methods section, presence of DH cytogenetics, ≥ 3 osteolytic lesions, IgD myeloma

Table 1 Baseline demographics and disease characteristics

Characteristic No. (%)	Total NDMM *N* = 618	sEMD group *N* = 77 (12.5%)	Non- sEMD group *N* = 541 (87.5%)	*P* value
Age, years				
Median (range)	62 (26–84)	62 (26–84)	63 (36–80)	0.631
Age ≤ 65, No. (%)	387 (62.6%)	48 (62.3%)	339 (62.7%)	0.956
Gender, No. (%)				0.294
Male	351 (56.8%)	48 (62.3%)	303 (56.0%)	
Female	267 (43.2%)	29 (37.7%)	238 (44.0%)	
ISS stage, No. (%)				0.184
Stage1	211 (34.1%)	22 (28.6%)	189 (34.9%)	
Stage2	250 (40.5%)	29 (37.7%)	221 (40.9%)	
Stage3	157 (25.4%)	26 (33.8%)	131 (24.2%)	
R-ISS stage, No. (%)				0.078
Stage1	104 (20.8%)	10 (15.4%)	94 (21.6%)	
Stage2	336 (67.2%)	42 (64.6%)	294 (67.6%)	
Stage3	60 (12.0%)	13 (20.0%)	47 (10.8%)	
Cytogenetics, No. (%)				
Gain/amplification of 1q21	266 (53.5%)	42 (65.6%)	224 (51.7%)	0.038
del(17p)	53 (10.7%)	10 (15.6%)	43 (9.9%)	0.168
t(11;14)	76 (16.6%)	9 (14.1%)	67 (17.0%)	0.563
t(4;14)	73 (15.6%)	14 (21.5%)	59 (14.6%)	0.155
t(14;16)	5 (1.1%)	1 (1.7%)	4 (1.0%)	0.635
Double-hit cytogenetics	77 (17.1%)	20 (34.5%)	57 (14.6%)	< 0.001
Baseline features				
Hypercalcemia, No. (%)	37 (6.0%)	10 (13.0%)	27 (5.0%)	0.006
Renal dysfunction (Ccr < 60 ml/min), No. (%)	159 (25.7%)	22 (28.6%)	137 (25.3%)	0.542
Anemia (< 100 g/L), No. (%)	297 (48.1%)	38 (49.4%)	259 (47.9%)	0.808
Osteolytic lesions (≥ 3 lesions), No. (%)	362 (58.6%)	64 (83.1%)	298 (55.1%)	< 0.001
LVEF (≥ 60%), No. (%)	570 (96.1%)	73 (97.3%)	497 (95.9%)	0.561
NT-proBNP, median (range) (ng/ml)	134 (5–32,606)	189 (21–11,328)	128 (5–32,606)	0.155
ECOG Score 0–2, No. (%)	544 (90.5%)	69 (92.0%)	475 (90.3%)	0.639
Elevated LDH, No. (%)				0.083
Yes	130 (21.0%)	22 (28.6%)	108 (20.0%)	
No	488 (79.0%)	55 (71.4%)	433 (80.0%)	
EMD at diagnosis, No. (%)				
ALL EMD	149 (24.1%)	32 (41.6%)	117 (21.6%)	< 0.001
EM-B (bone-related)	121 (19.6%)	20 (26.0%)	101 (18.7%)	0.175
EM-E (extraosseous)	28 (4.5%)	12 (15.6%)	16 (3.0%)	< 0.001
Induction regimen, No. (%)				
PI-based	486 (78.6%)	61 (79.2%)	452 (78.6%)	0.433
IMiD-based	51 (8.3%)	5 (6.5%)	46 (8.5%)	0.705
PI and IMiD combination	57 (9.2%)	6 (7.8%)	51 (9.4%)	0.800
PI, IMiD and CD38 Ab combination	20 (3.2%)	4 (5.2%)	16 (3.0%)	0.488
ASCT, No. (%)				0.010
Yes	235 (38.0%)	19 (24.7%)	216 (39.9%)	
No	383 (62.0%)	58 (75.3%)	325 (61.0%)	
M type, No. (%)				
IgA	145 (23.5%)	20 (26.0%)	125 (23.1%)	0.819
IgG	309 (50.1%)	40 (51.9%)	269 (49.8%)	0.687
IgD	23 (3.7%)	6 (7.8%)	17 (3.1%)	0.090
sFLC	125 (20.3%)	9 (11.7%)	116 (21.5%)	0.064
Non-secretory	15 (2.4%)	2 (2.6%)	13 (2.4%)	1.000

LVEF left ventricular ejection fraction, *NT-proBNP* N-terminal pro-B-type natriuretic peptide, *ECOG* Eastern Cooperative Oncology Group

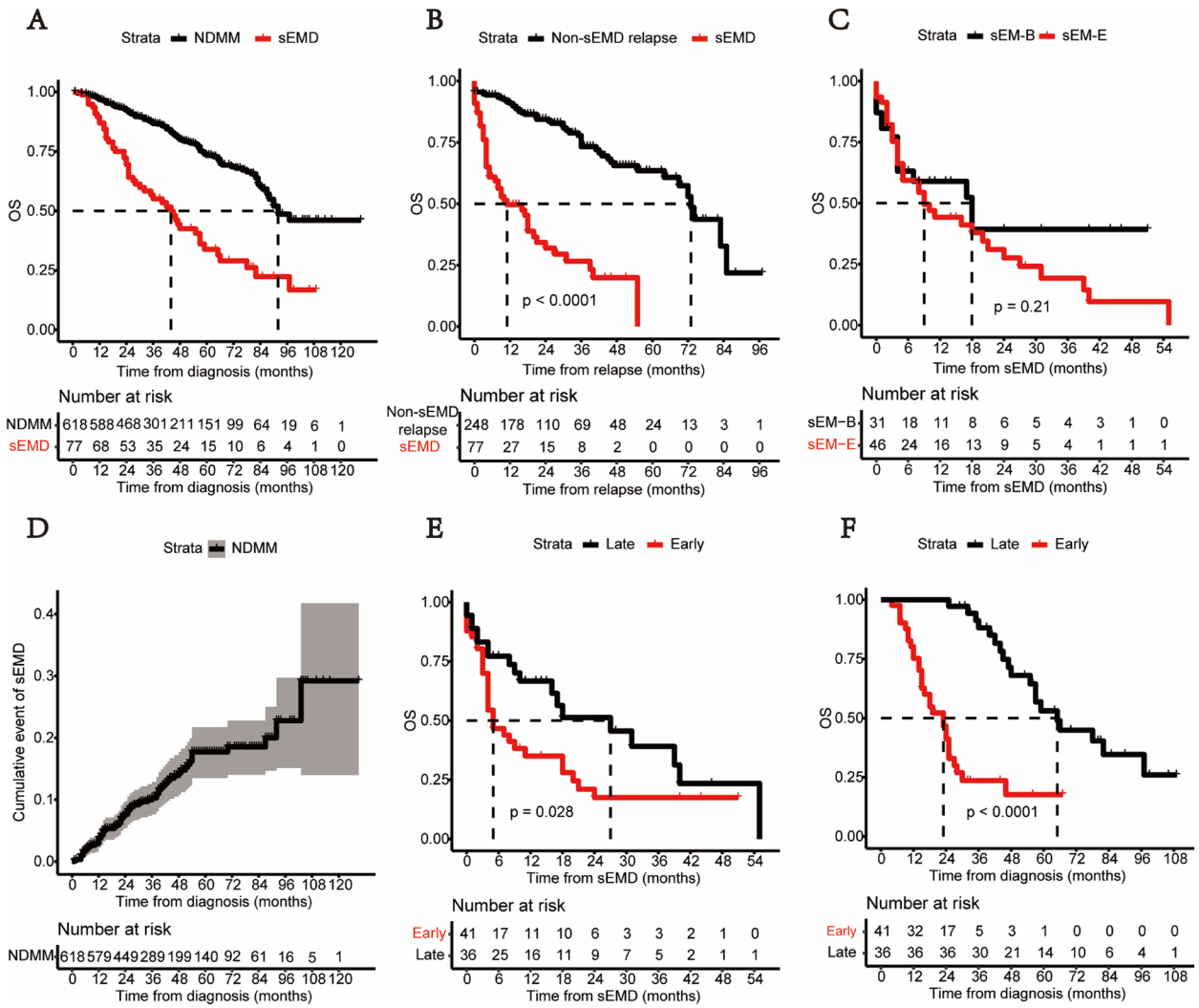

Fig. 1 Survival outcomes and sEMD relapse patterns in newly diagnosed and relapsed MM patients. **A** OS from MM diagnosis for the entire cohort of 618 patients and patients with sEMD. **B** Post-relapse OS for patients stratified by the presence of sEMD. **C** Post-sEMD OS for patients stratified by sEM-B or sEM-E. **D** Cumulative incidence of sEMD development for the entire cohort of 618 patients. **E** OS from the onset of sEMD for early versus late sEMD. **F** OS from MM diagnosis for early versus late sEMD

subtype, and non-ASCT contributed 1 point each, and EM-E subtype contributed 2 points. The distribution of patients with different sEMD predictive scores was shown in Table 2. Corresponding 3-year sEMD relapse rates for each score group were 0%, 6.1%, 9.9%, 45.5%, 87.5%, and 60.0%, respectively (Fig. 3A). Risk stratification based on the scoring system categorized 390 patients (87%) as low risk (0–2 points) and 59 patients (13%) as high risk (3–5 points), as per Table 2. A significant disparity in 3-year sEMD relapse rates between low vs high-risk groups (6.6% vs 52.8%, $p < 0.001$) (Fig. 3B) supports the discriminative power of the scoring system in NDMM. Furthermore, the estimated 5-year OS prognosis was considerably poorer for high-risk sEMD subjects (22.6% vs 76.8%, $p < 0.001$, Additional file 1: Fig. S1). The robustness of the sEMD predictive model was also demonstrated across various patient subsets, including ASCT (Additional file 1: Fig. S2A), non-ASCT recipients (Additional file 1: Fig. S2B), and patients with different induction regimens (though the IMid induction subgroup was excluded due to lack of statistical significance, it still exhibited a clear trend toward high-risk patients being more prone to sEMD), indicating its broad applicability in clinical practice (Additional file 1: Fig. S3A-D). Notably, combination induction therapies, such as PI + IMid or PI + IMid + CD38Ab, were associated with a reduced incidence of sEMD relapse in low-risk groups, in contrast to regimens consisting solely of a PI or an IMiD (Additional file 1: Fig. S3 A–D, black curves). However, combination induction failed to show a decrease in sEMD rate

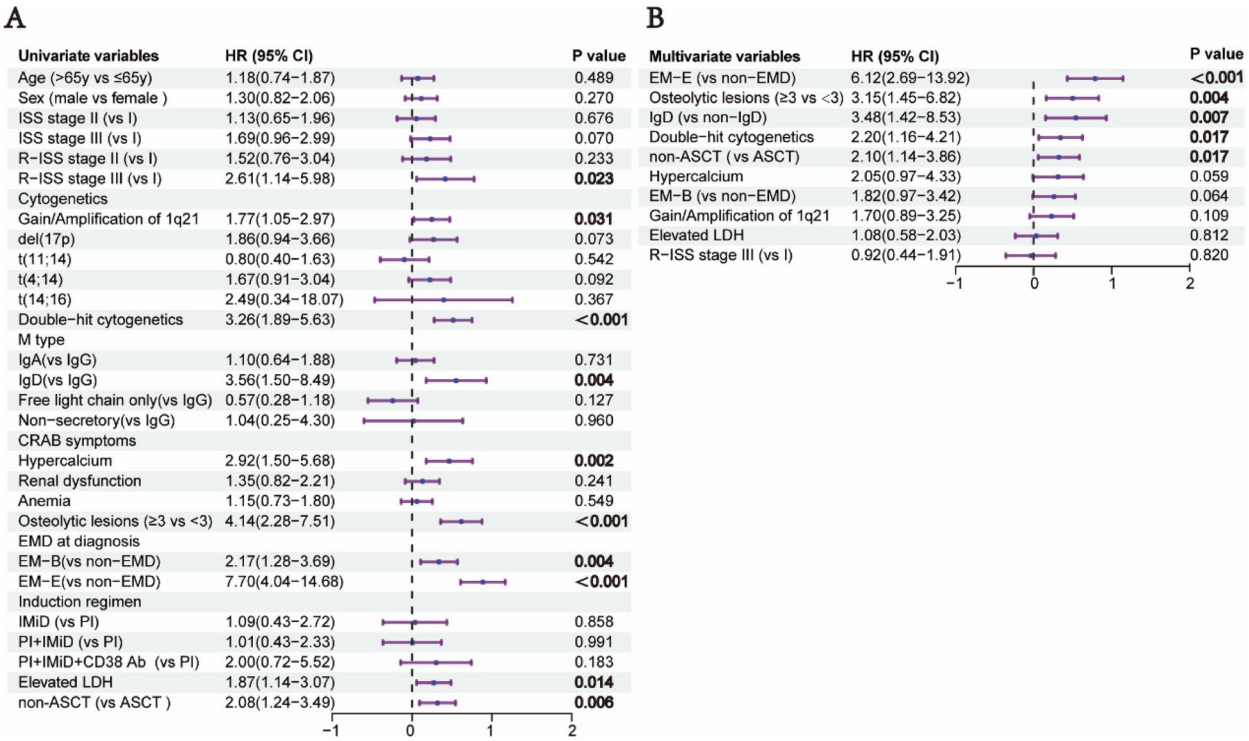

Fig. 2 DH cytogenetics as a significant predictor of sEMD relapse in time-dependent univariate and multivariate analyses. **A** Univariate Cox regression analysis identifying significant predictors of sEMD relapse. **B** Multivariate Cox regression analysis identifying significant predictors of sEMD relapse. HR, hazard ratio; CI, confidence interval; HR was transformed into log10(HR) in the figure

in high-risk groups (Additional file 1: Fig. S3 A–D, red curves). Additional file 1: Table S2 details the occurrence of individual risk features within each categorized risk group, highlighting that 57.6% of high-risk sEMD individuals possessed DH cytogenetics, and 33.9% had EM-E at diagnosis. Furthermore, the model-based nomogram serves as an excellent complement to the five-predictor scoring system, enabling a quick and precise estimation of sEMD risk (Additional file 1: Fig. S4).

Single-cell profiling delineated biological traits of DH myeloma cells

To elucidate transcriptional states in newly diagnosed DH myeloma at single-cell resolution, we analyzed publicly available scRNA-seq data from bone marrow mononuclear cells of eight healthy individuals and five DHMM patients, including three with concurrent 1q + and t(4;14) abnormalities, one with 1q + and 17p − abnormalities, and one with 1q + and t(14;16) abnormalities. We acquired single-cell transcriptomic profiles for 66,092 high-quality mononuclear cells, identifying six major cell types—T/NK cells, plasma cells, myeloid cells, B cells, and precursor cells—using established marker genes (Fig. 4A, B). UMAP analysis distinctly separated immune cells from plasma/myeloma cells, with high plasma cell (PC) scores (Fig. 4C). Our analysis encompassed 18,099 plasma cells and 47,993 immune cells, enabling an integrated examination of tumor and immune cell heterogeneity in DHMM. Further re-clustering of plasma cells revealed 10 distinct clusters, uncovering significant transcriptional disparities across patients (Fig. 4D, left). Notably, normal plasma cells (nPCs) from healthy individuals and a minor subset of MM cells co-clustered in cluster 7, indicating the presence of nPCs within MM samples (Fig. 4D, right). Hierarchical clustering based on gene expression linked DH myeloma cells to transcriptional subtypes that corresponded with their cytogenetic profiles, underscored by driver gene expression (Fig. 4E). GSEA identified significant upregulation of biological processes in DH myeloma cells (Fig. 4F), such as oxidative phosphorylation, cell cycle, hypoxia-inducible factor 1 (HIF-1) signaling, proteasome, and mitogen-activated protein kinase (MAPK) activation, all of which have been linked to the progression of sEMD in previous reports [24, 25]. Additionally, pathways related to nuclear export and DNA methylation were upregulated in DH cells, presenting potential therapeutic targets for treating DH myeloma [26] and preventing sEMD [25]. Immune response alterations in DH cells included enhanced type I interferon production, known to promote Treg expansion

Table 2 sEMD Score definition and high-risk group classification

Risk feature	sEMD HR (95% CI)	Score value		
DH cytogenetics	2.20 (1.16–4.21)	1		
EM-E at diagnosis	6.12 (2.69–13.92)	2		
≥ 3 osteolytic lesions	3.15 (1.45–6.82)	1		
Non-ASCT	2.10 (1.14–3.86)	1		
IgD subtype	3.48 (1.42–8.53)	1		
Total score	No (%)	Group (total score)	No (%)	Median time to sEMD
0	60 (13%)	Low (0–2)	390 (87%)	-
1	166 (37%)			
2	164 (37%)			
3	46 (10%)	High (3–5)	59 (13%)	36 months
4	8 (2%)			
5	5 (1%)			

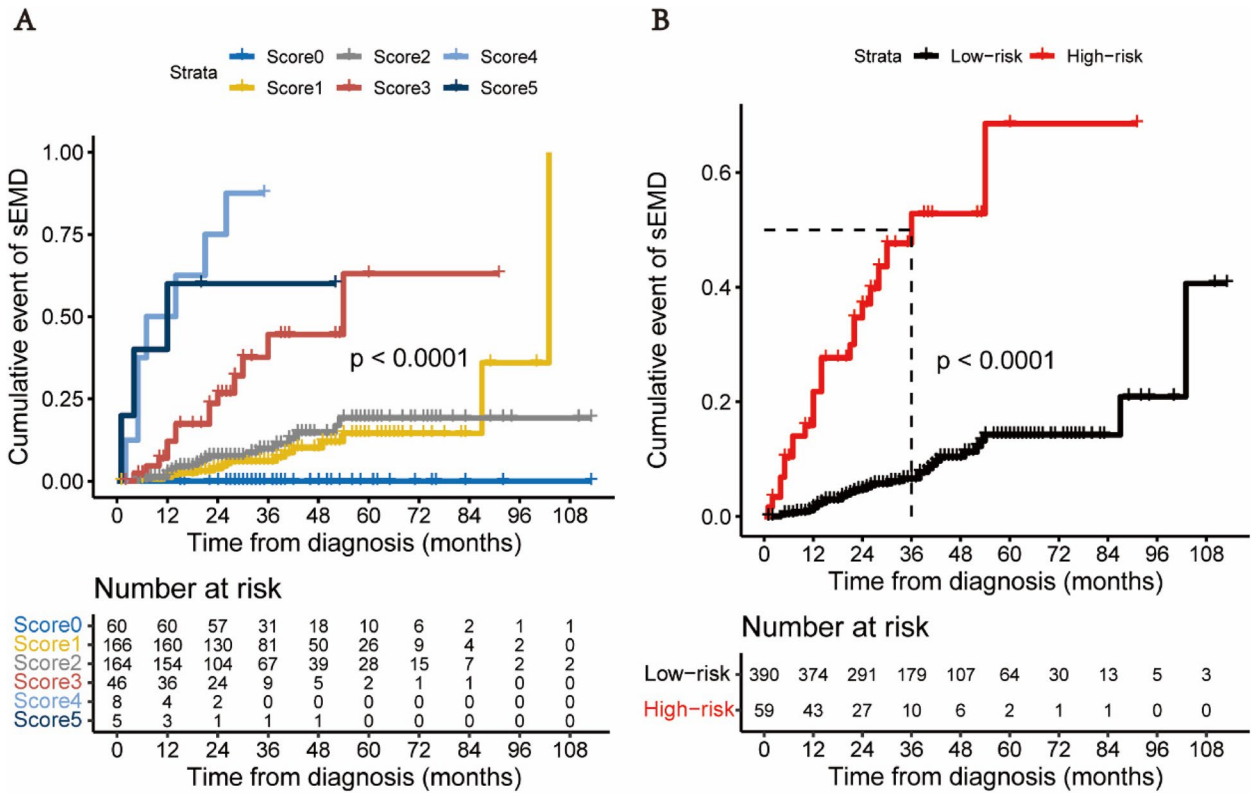

Fig. 3 Cumulative incidence of sEMD. **A** The cumulative incidence of sEMD stratified by additive scores. **B** The cumulative incidence of sEMD stratified by low (0–2 points) versus high (3–5 points) sEMD risk

and immunosuppression [27]. Compared to nPCs, DH myeloma cells significantly overexpressed genes involved in anti-apoptosis/drug resistance: B-cell lymphoma 2 (BCL2), myeloid cell leukemia 1 (MCL1), peptidylprolyl isomerase A (PPIA) [21], poly (ADP-ribose) polymerase 1 (PARP1) [28]; metastasis: C–C motif chemokine ligand 3 (CCL3) [29], Y-box binding protein 1 (YBX1) [30], F-box and WD repeat domain-containing 7 (FBXW7) [31], stathmin 1 (STMN1) [32]; and key therapeutic targets: tumor necrosis factor receptor superfamily member 17 (TNFRSF17), G-protein-coupled receptor class C group 5 member D (GPRC5D), CD38, enhancer of zeste homolog 2 (EZH2) [25] (Fig. 4G). These findings underscore the biological characteristics of DH myeloma cells possibly involved in sEMD development and propose potential therapeutic strategies.

Single-cell analysis revealed dysfunctional immune cell features of DH patients in the bone marrow environment

We conducted an integrative analysis combining the CD138-negative fraction from DHMM patients and healthy controls into a comprehensive dataset, uncovering 18 distinct cell types encompassing all principal mononuclear bone marrow cells and progenitor cells leading to myeloid/dendritic and B-cell lineages, as well as segregated T/NK-cell groups (Fig. 5A, B). Comparative analysis between DHMM patients and healthy subjects revealed marked differences in cell-type distribution (Fig. 5C, D), confirming a reduction in CD8 + naive cells and the B-cell lineage [33], alongside an increase in CD16 + monocytes and Treg cells [20]. While immature NKbright cells showed a marginal increase, NKdim effector cell populations increased in abundance [20]. Interestingly, no increase was observed in effector T-cell populations, including CD8 + memory and cytotoxic cells.

In our study, we explored the bidirectional influences between myeloma and bone marrow environment (BME) cells in DHMM, mediated by cytokines and their receptors, which are crucial in disease progression and treatment resistance [34]. By evaluating ligand-receptor pair expressions, we discerned enhanced interactions of DH myeloma cells with the myeloid lineage (CD14 + / CD16 + monocytes and dendritic cells), CD8 + T cells, and NK cells (Fig. 5E). Focusing on interactions markedly increased in DHMM compared to healthy individuals, we detected elevated expression of pro-inflammatory cytokines in DH cells (Fig. 5F). These include CCL3 [29],

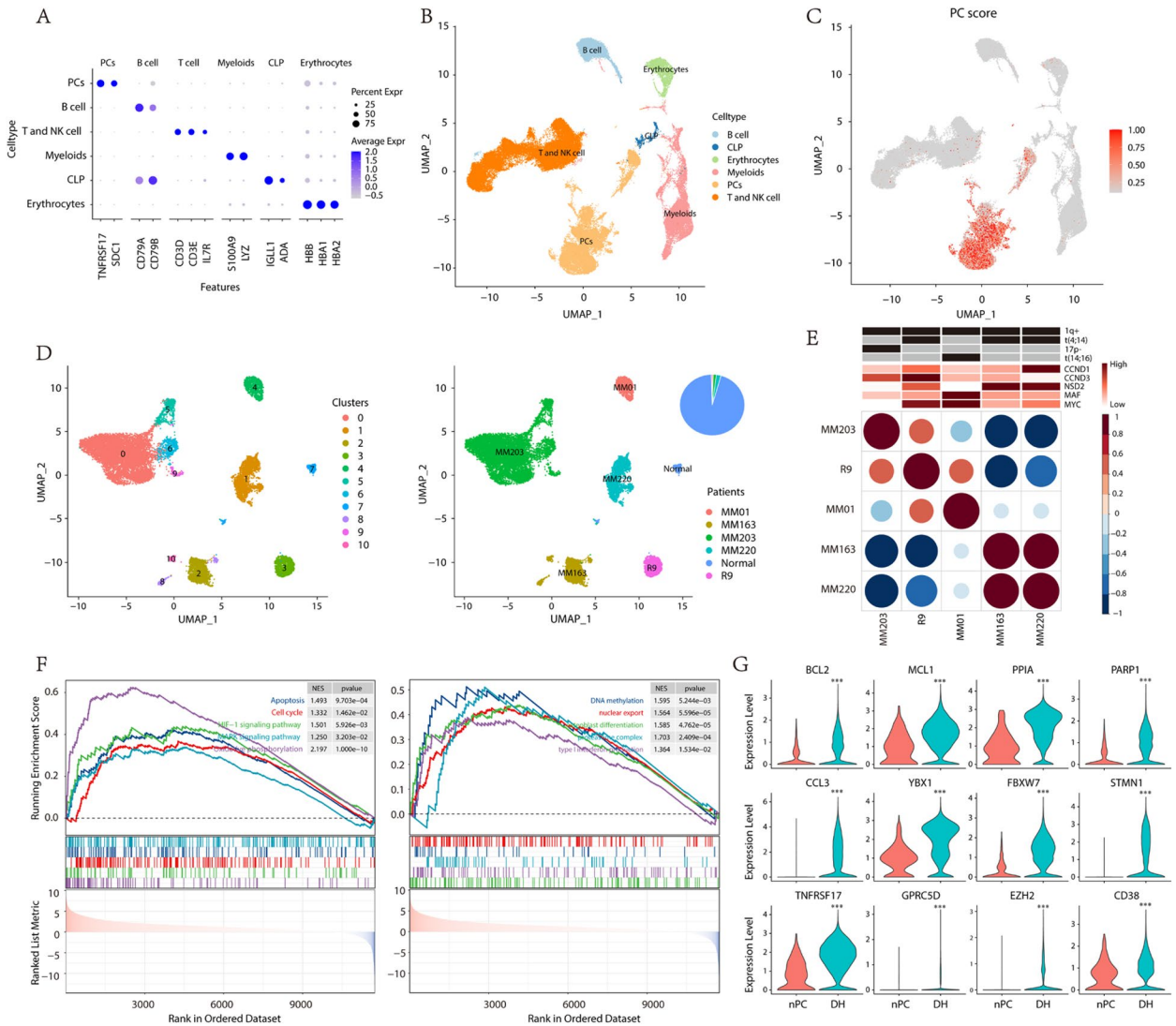

Fig. 4 Single-cell analysis identified the biological features of double-hit (DH) myeloma cells with the propensity to progress to extramedullary disease. **A** Gene expression dot plot of major marker genes for individual cell types. **B** UMAP plot displaying single cells colored by major cell types. **C** UMAP plot showing single cells colored by plasma cell (PC) score based on the expression of the plasma cell markers (SDC1/CD138, TNFRSF17 and SLAMF7). **D** UMAP plots demonstrating re-clustering of plasma cell (PC) colored by subclusters (left) and patients (right). The pie chart inset shows the nPCs fraction colored according to patient. **E** Pearson correlation matrix illustrating averaged gene expression levels per patient, with cytogenetic information on top and averaged expression of five MM driver genes at the bottom. **F** GSEA results displaying significant positive enrichment of biological processes and signaling pathways in DH myeloma cells. **G** Comparisons of selected gene expression related to apoptosis/drug resistance, metastasis, and clinically important targets between normal plasma cells and DH myeloma cells

macrophage migration inhibitory factor (MIF) [35], insulin-like growth factor 1 (IGF1) [36], and midkine (MDK) [37], with corresponding receptors predominantly found in myeloid and dendritic cells. Additionally, DH cells frequently upregulated adhesion G protein-coupled receptor E5 (ADGRE5), an adhesion receptor implicated in tumor invasiveness and metastasis, interacting primarily with CD55[38]. We also observed an increase in human leukocyte antigen (HLA)-I molecules such as HLA-E and HLA-F in DH cells, targeting inhibitory receptors killer cell lectin-like receptor subfamily C, member 1 (KLRC1) [39] and leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1) [40] respectively, which are expressed mainly in monocytes, NK, and dendritic cells. We further examined T/NK-cell populations for signs of exhaustion, characterized by upregulated inhibitory receptors following chronic antigen exposure. The highest expression of exhaustion markers was noted in Treg,

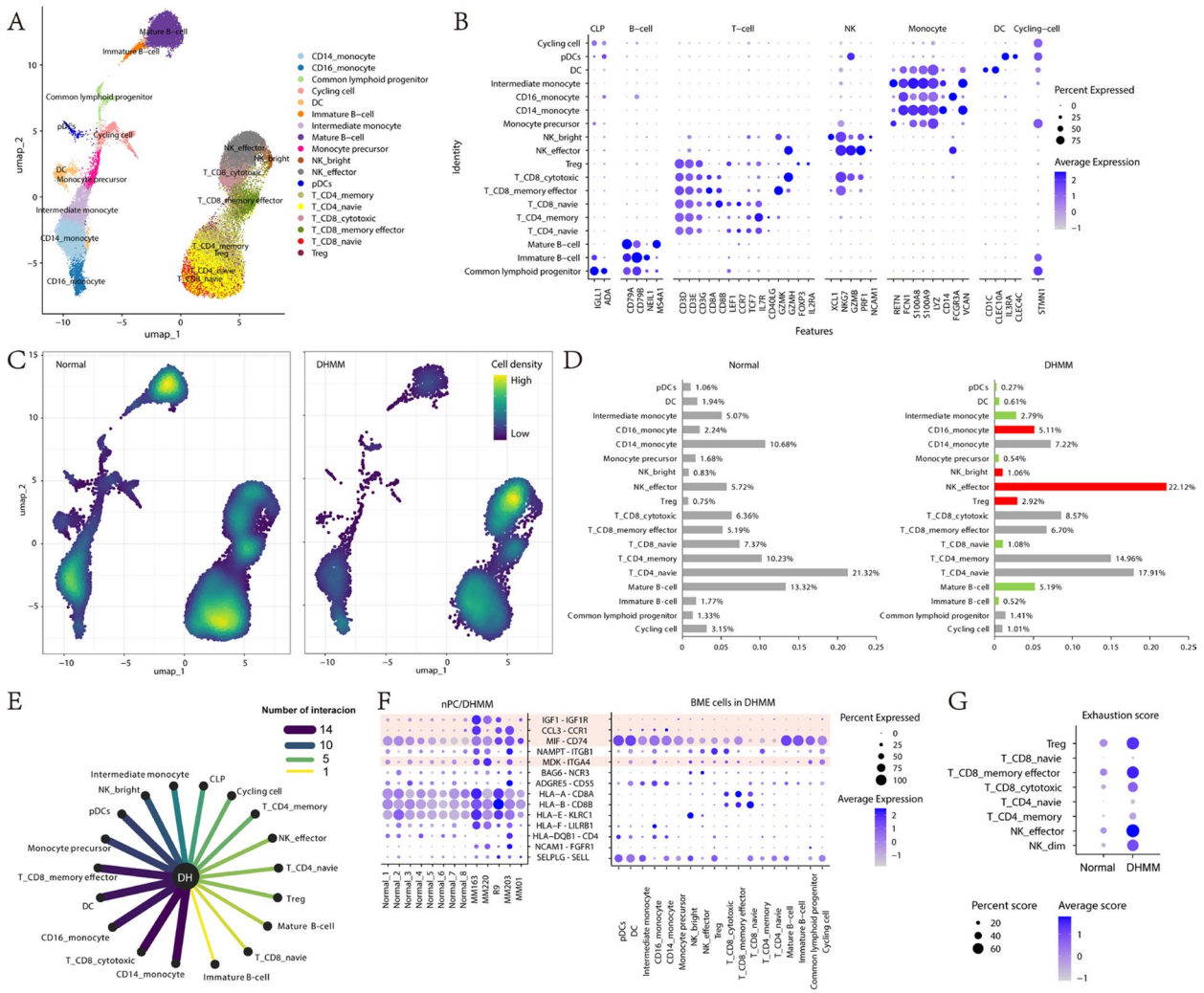

Fig. 5 Single-cell analysis revealed cellular interactions between DH myeloma and BME cells. **A** UMAP plot displaying CD138-negative BME cells colored by cell type, with schematic overview for spatial distribution of major cell types. **B** Gene expression dot plot of key marker genes for individual BME cell subsets. **C** Point-density UMAP plot comparing cell composition between healthy donors (left) and DHMM (right). **D** Bar plot showing cell type fractions in healthy donors (left) and DHMM (right) individually. Compared to nPC, the red bars represent a significant upregulation in proportion, while the green bars indicate a significant downregulation in DHMM. **E** Cellular interactions between DH myeloma and BME cell subsets based on ligand-receptor expression, ordered by detected interactions. **F** Dot plot illustrating the gene expression of ligand-receptor pairs. Left panel depicted expression in nPCs/DH myeloma cells, while the right panel showed expression in BME cell subsets. Only interactions that are upregulated between DH myeloma cells and immune cell types in comparison to nPCs are displayed. Inflammatory cytokine interactions are highlighted in pink. **G** Gene expression dot plot showing exhaustion signature score levels in T/NK-cell subpopulations between healthy donors and DHMM

CD8 + memory effector, and NK effector cells (Fig. 5G). Consistently, compromised immune function in these immune cells was reported during sEMD progression [24]. Overall, our analysis reveals that in DH patients, dysfunctional immune cell features may be driven by multiple transcriptionally overexpressed cytokines and surface markers in DH cells, potentially leading to an immunosuppressive BME.

Discussion

This study employed a time-dependent regression model to identify five predictors of sEMD, with a specific focus on one predictor: the DHMM myeloma cells and their associated TME, examined through scRNA-seq analysis. EMD manifests in two distinct clinical phenotypes: primary EMD, which occurs in previously untreated patients with MM, and secondary EMD (sEMD), found in individuals with RRMM. There is variation in the

criteria used to define EMD [41], particularly when classifying EM-B or EM-E. It is generally recognized that the prognosis for patients with primary EM-B is better than that for patients with EM-E, a finding that is supported both by our research [42]and others [43, 44]. However, this study demonstrated that in the context of sEMD, the OS did not significantly differ between the sEM-B and sEM-E groups ($p=0.210$), despite a trend toward an even worse survival for sEM-E, corroborated by findings from a previous report [44]. Consequently, sEM-B warrants increased clinical attention. For the purposes of subsequent analyses, sEM-B and sEM-E are considered collectively.

We observed a median 22-month interval from initial MM diagnosis to sEMD development in 77 sEMD patients, aligning with 22.2 months reported in a recent publication [25]. Our study sought to elucidate risk factors for sEMD and yielded three significant insights: (i) Analysis indicated that patients with early sEMD relapse ($\leq$22 months post-MM diagnosis) experienced significantly inferior OS following sEMD relapse (5.0 months). Thus, our study introduces a time-dependent approach to predicting sEMD risk, differing from previous studies that utilized a logistic regression model [6, 45]. (ii) The research included variables such as EMD presence at diagnosis—distinguishing between EM-B and EM-E—as well as cytogenetics identified by FISH analysis, IgD myeloma subtype, and ASCT status, among others. Five independent prognostic factors were identified, with different weights assigned to each predictor based on their respective coefficients. (iii) We proposed for the first time that the DH cytogenetics at diagnosis independently predict the development of sEMD.

Previous reports on risk factors for sEMD in MM are limited, yet identified risks show both concordance and divergence with our findings, potentially due to differences in variables included and statistical methods employed. Similar to previous studies [7, 46], our findings reveal that novel induction regimens do not induce sEMD. Furthermore, our data suggest that the utilization of multiple novel agents in combination, which employ diverse mechanisms of action, may confer a protective effect against the development of sEMD in patients classified as low-risk (Additional file 1: Fig. S3). We corroborate the association of extensive osteolytic lesions with increased sEMD risk as others have described [6, 45], suggesting that the disruption of bone integrity may facilitate the egress of myeloma cells from the marrow to extramedullary sites. This supports the observed survival benefit conferred by bisphosphonate therapy and denosumab [47, 48]. According to IMWG guidelines [49], zoledronic acid is to be recommended monthly for a minimum of 12 months until reaching at least VGPR. Denosumab can also be considered, particularly in patients with renal impairment. Given that 52.8% of patients with high-risk sEMD manifest sEMD within 3 years, we recommend prolonged bisphosphonate or denosumab therapy beyond this period to strengthen skeletal integrity in this subset.

While EMD at diagnosis has been shown as a predictor for sEMD [45, 50], the significance of EM-B involvement at diagnosis is not fully elucidated. Our analysis revealed EM-B at diagnosis as a significant univariate predictor of sEMD, yet it did not retain significance in the multivariate context. Consistently, our prior research corroborates ASCT can overcome the negative prognostic effect of EM-B [42], and the current study reinforces ASCT as a prophylactic factor against the sEMD. Furthermore, the nomogram clearly identifies EM-E at diagnosis as the most heavily weighted independent predictor of sEMD, whose influence persisted even after adjustment for high-risk cytogenetics, ASCT, M subtype, and elevated LDH levels. Consistent with our prior reports of markedly inferior survival (median OS of 25.6 months) in EM-E at diagnosis [42], this observation suggests a unique pathobiology underpinning drug resistance and metastatic potential in primary EM-E, warranting further investigation. In our research, IgD has been identified as an independent prognostic factor for sEMD, consistent with the previous findings that sEMD is associated with IgD subtype [45, 50]. Although IgD myeloma represents only 1–2% of all NDMM cases, it is associated with shortened OS, with durations reported between 2 and 3 years [51, 52]. The IgD subtype accounts for 3.7% of our study population, with a higher prevalence of 7.8% within the sEMD group. Therefore, given the poor survival and the increased propensity for sEMD relapse, patients with IgD should be considered high-risk and treated accordingly. Different from others [2, 6], our data did not indicate a predisposition for younger patients to develop sEMD. The discrepancy might be attributable to the greater percentage of younger patients receiving ASCT, which serves as a sEMD-protective predictor in our cohort, compared to the older (58.4% vs 3.9%).

The relationship between cytogenetic abnormalities and the occurrence of sEMD remains ambiguous. Zanwar et al. identified an independent association between the presence of 1q+and sEMD, compared to controls without sEMD who were matched based on the date of MM diagnosis [2]. Consistently, we also observed this association; however, 1q+did not remain an independent predictor upon adjustment for ASCT and DH cytogenetics in the multivariate results. This could be in line with our previous study demonstrating the potential of ASCT to negate the adverse prognostic impact of single 1q+[15], and the fact that 29.9% of patients with 1q+in

the current study also harbored other high-risk chromosomal aberrations. This study distinguishes itself from other models [2, 6, 45] by considering the concurrence of multiple high-risk CAs, which was not uncommon (17.1%) in our patient population. The significance of DH cytogenetics in sEMD risk was firstly noted when several DH patients, notably three with co-existing 1q + and t(4;14) and one with 1q + and 17p −, developed sEMD and subsequently succumbed post-transplant in our previous report [15]. Adding to this, this study further demonstrated DH at diagnosis as a robust predictor of sEMD development in both univariate and multivariate models. In our prediction model, high-risk sEMD patients with a total score of 3–5 included those DH with extensive bone lesions, who did not undergo ASCT, or those EM-E at diagnosis with an IgD subtype. For those with an ultra-high-risk sEMD scoring 4–5, such as DH patients who present with both EM-E and extensive bone damage at diagnosis, there is a 50% probability of sEMD manifestation within 1 year, irrespective of ASCT intervention. Considering the grim outlook for high-risk sEMD, there is a pressing necessity to not only improve post-sEMD survival but also to formulate preventive therapeutic approaches.

Furthermore, we are curious about the mechanisms driving sEMD, focusing on scRNA-seq analysis of newly diagnosed DH specimens. This study corroborates earlier findings [2, 6], demonstrating that 16% of NDMM patients with 1q21 + progress to sEMD. 1q21 + status alone does not independently predict sEMD development, which instead results from the interaction between 1q21 + and MAPK pathway mutations [25], associated with MAPK activation [53]. Although mutation data were incomplete for our cohort, the observation that 94% (72/77) of DH patients exhibited 1q21 + abnormalities in our study, coupled with the demonstration of MAPK pathway upregulation in DH samples via scRNA-seq, suggests a biological predisposition towards this interaction. Additionally, we observed an immunosuppressive microenvironment in DH patients characterized by the exhaustion of CD8 + T and NK effector cells, which aligned with prior studies identifying them as key immune components in the extramedullary microenvironment [25] and implicating their exhaustion in the development of sEMD [24]. This study aimed to identify high-risk sEMD patients at diagnosis for timely and effective intervention. Within this group, DH patients represent a subset that can be readily identified through standard FISH analysis. Though we have previously documented the benefits of achieving MRD negativity within 3 months post-transplant for DH patients, this alone is not sufficient for a long-term favorable prognosis [15]. Aiming for sustained MRD negativity at a higher sensitivity threshold might be a strategy to counter the adverse outcomes [13]. scRNA-seq analysis highlighted upregulated targets and activated signaling pathways in DH samples, suggesting that a comprehensive treatment strategy that includes PI, IMiDs, CD38Ab, Selinexor (an oral exportin 1 inhibitor), and immunotherapy targeting B-cell maturation antigen (BCMA) and/or GPRC5D could be employed to eliminate residual myeloma cells as effectively as possible. We believe that this approach should be extended to those at high risk for sEMD, who may be eligible for intensified treatment regimens incorporating pioneering treatments such as CART and bispecific T-cell engagers as front-line therapies. As demonstrated in our previous LEGEND-2 study [54–57], BCMA-targeting CART (LCAR-B38M) is potentially efficient, achieving 67.6% MRD-negativity and 5-year OS of 49.1% in RRMM who had received ≥ 3 lines of prior therapy [57]. Notably, Jelinek et al. reported decreased expression of therapeutically relevant targets such as CD38, SLAM family member 7 (SLAMF7), GPRC5D, and Fc receptor homolog 5 (FcRH5) in extramedullary myeloma cells, with no change in BCMA expression, based on scRNA-seq comparisons between extramedullary myeloma cells at relapse and bone marrow myeloma cells at diagnosis [25]. This observation partially supports our finding that a sEMD patient with liver involvement in our LEGEND-2 study [55] survived for 54 months after BCMA-CART therapy, significantly longer than typical sEMD patients. Moreover, Jelinek et al. also highlighted the upregulation of methylation-associated genes CD70 and EZH2 in extramedullary myeloma cells [25], identifying potential therapeutic targets. Our research similarly identified methylation pathway activation and elevated EZH2 levels in DH samples at diagnosis, indicating that integrating demethylation agents into frontline therapy for DHMM patients to possibly prevent sEMD merits further exploration. Additionally, while CD38 expression decreases in extramedullary myeloma, CD38Ab can target CD38 + immunosuppressive cells, such as Tregs and NK cells [58]. Despite BME cells constituting a mere 10% in extramedullary myeloma [25], the strategic exclusion of CD38Ab from sEMD treatment protocols requires careful consideration, especially since certain combination therapies, like those with EZH2 inhibitors, can enhance CD38 expression on myeloma cells [59].

This study is limited by the small number of scRNA-seq samples from DH patients and the absence of mutation data, restricting a comprehensive understanding of DH myeloma pathogenesis. Our findings identify promising targets and pathways that may underlie the propensity

of DH myeloma cells to progress to sEMD, yet further experimental validation is necessary to elucidate these mechanisms. Other important limitations include the absence of comorbidity assessment, frailty evaluation, and socio-demographic prognostic factors. Furthermore, the percentage of patients treated with the combination of PI/IMiD is low due to insurance coverage constraints.

Conclusions

Moving forward, more prospective studies including a baseline and dynamic comprehensive clinical, epidemiological, and genomic prognostic assessment are warranted to define strategies for sEMD prevention and unveil potential tailored therapeutic approaches. Echoing traditional Chinese medical philosophy, "the supreme doctor treats before the disease manifests," our predictive model for sEMD, informed by scRNA-seq of DH samples, offers prospective insights for the potential prediction and prevention of sEMD.

Abbreviations

ADGRE5	Adhesion G protein-coupled receptor E5
ASCT	Autologous stem-cell transplantation
BCL2	B-cell lymphoma 2
BCMA	B-cell maturation antigen
BME	Bone marrow environment
CCL3	C-C motif chemokine ligand 3
CART	Chimeric antigen receptor T-cell
CI	Confidence interval
DH	Double-hit
EZH2	Enhancer of zeste homolog 2
FcRH5	Fc receptor homolog 5
FBXW7	F-box and WD repeat domain-containing 7
FISH	Fuorescence in-situ hybridization
GPRC5D	G protein-coupled receptor class C group 5 member D
GSEA	Gene set enrichment analysis
HIF-1	Hypoxia-inducible factor 1
HLA	Human leukocyte antigen
HR	Hazard ratio
IGF1	Insulin-like growth factor 1
IMWG	International Myeloma Working Group
KLRC1	Killer cell lectin-like receptor subfamily C, member 1
LILRB1	Leukocyte immunoglobulin-like receptor subfamily B member 1
MAPK	Mitogen-activated protein kinase
MDK	Midkine
MCL1	Myeloid cell leukemia 1
MM	Multiple myeloma
MIF	Macrophage migration inhibitory factor
nPCs	Normal plasma cells
NDMM	Newly diagnosed multiple myeloma
OS	Overall survival
PARP1	Poly (ADP-ribose) polymerase 1
PPIA	Peptidylprolyl isomerase A
PFS	Progression-free survival
RRMM	Relapsed/refractory multiple myeloma
sEM-B	Secondary bone-related extramedullary
sEM-E	Secondary extraosseous extramedullary
sEMD	Secondary extramedullary myeloma disease
SLAMF7	SLAM family member 7
STMN1	Stathmin 1
TNFRSF17	Tumor necrosis factor receptor superfamily member 17
UMAP	Uniform manifold approximation and projection
YBX1	Y-box binding protein 1

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04086-y.

Additional file 1: Table S1- [Involved sites of sEMD relapse]. Figures S1-S3. Fig. S1- [OS prognosis of NDMM in high and low risk sEMD groups]. Fig. S2- [sEMD cumulative incidence in ASCT and non-ASCT patient subsets]. Fig. S3- [sEMD cumulative incidence in patients with different induction regimens]. Table S2- [Risk feature distribution by sEMD risk group]. Figures S4- [sEMD incidence nomogram].

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Authors' contributions

Y.T. and J.M. designed the study. Y.T., S.J., and Z.W. analyzed the data and wrote the paper. Y.T., S.J., Z.W., M.P., W.O., J.X., Y.W., and Y.L. participated in the patient data collection. Y.W., Y.L., W.Z., and J.L. provided the consultancy, corrections, and comments. J.M. supervised the research and provided the foundations. All authors contributed to manuscript revision and correction and approved the submitted version.

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Data availability

Public health data from eight normal individuals were obtained from the R package SeuratData:hcabm40k. Public scRNA-seq datasets for multiple myeloma were downloaded from the GEO database under accession numbers GSE117156 [18], and GSE189460 [19], and GSE124310 [20]. Additional datasets generated during and/or analyzed during the current study are available in the Figshare repository, with the identifier (https://doi.org/10.6084/m9.figshare.28587827).

Declarations

Ethics approval and consent to participate

The Internal Review Board (IRB) of Ruijin Hospital approved this study (Approval number 2014–059). Informed consent was obtained from all study participants. The study was carried out following the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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