Am J Transl Res 2020;12(6):2425-2438
www.ajtr.org /ISSN:1943-8141/AJTR0108428

Original Article

A centromere-associated gene score for rapid determination of risk in multiple myeloma

Hua Bai[1*], Peipei Xu[1*], Lili Wang[2*], Bing Chen[2]

[1]*Department of Hematology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing 210008, Jiangsu, China;* [2]*Department of Hematology, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, Nanjing 210008, Jiangsu, China.* [*]*Equal contributors.*

Received January 28, 2020; Accepted May 25, 2020; Epub June 15, 2020; Published June 30, 2020

Abstract: Risk stratification in patients with multiple myeloma (MM) remains a challenge. As clinicopathological characteristics have been proven deficient for accurately defining risk stratification, molecular markers have gradually become the focus of interests. This study investigated the expressions of centromere-associated genes in MM patients, their potential as prognostic markers, and their roles in disease progression. Several cohorts of 2301 MM patients were enrolled and gene expression profiling (GEP) was used to screen for *CENP-A* through *CENP-W*. Correlations between centromere-associated genes and clinicopathological characteristics, proliferative activity and recurrence of MM patients were analyzed. Clinically, *CENP-E/H/K/L/N/U/W* expressions were present at high-risk MM, which were even stronger elevated in patients with high tumor burden and recurrence. Mechanistically, *CENP-E/H/K/L/N/U/W* and *FOXM1* were positively expressed in MM patients, which play synergistic or additive effects in clinical outcome. Furthermore, *CENP-E/H/K/L/N/U/W* were used to construct a centromere-associated gene score (CGS) model, which proved to be strongly prognostic values in several independent cohorts compared to usual clinical prognostic parameters using multivariate Cox analysis. Patients in the CGS low-risk group were significantly related to better clinical outcome than those in high-risk group. In this study, we provided proof-of-concept that *CENP-E/H/K/L/N/U/W* have critical roles in MM patients' progression and prognosis. The CGS model validated in different datasets clearly indicated novel risk stratification for personalized anti-MM treatments.

Keywords: Multiple myeloma, risk stratification, centromere

Introduction

Multiple myeloma (MM) is plasma cell malignancy that proliferates in the bone marrow, and the progressive plasma cell is characterized with recurrent gene translocations, deletions or gains and changes [1-3]. Gene expression signatures made it possible to identify gene expression in myeloma cell linked with progression free and/or overall survival (PFS/OS) of MM patients. Zhan *et al.* identified 8 genetic subgroups of MM [4]; Subsequently, Shaughnessy *et al.* established a 70-gene risk scoring system able to divide 13% of MM cases into high-risk group [5]; Later, Decaux *et al.* developed an IFM15 risk stratification, which classify 25% of MM cases as high-risk [6]. These risk scoring systems included abundant genes coding for proteins involved in multistep processes of universal aneuploidy and recurrent chromosomal aberrations, which is considered to be associated with chromosomal instability (CIN). CIN can allow the rapid accumulation of changes that promote myeloma progression, growth and heterogeneity, and contribute to intrinsic and acquired drug resistance [7, 8]. Therefore, CIN-related biomarkers that can predict the incidence of progression and/or recurrence are clinical priority for MM risk stratification.

The exact causes of CIN in most cancers remain unclear. Proposed mechanisms include oncogene-induced replication stress, breakage-fusion-bridge cycles induced by telomere translocations, dysfunction and aberrant mitosis [9]. Another important mechanism involves centromeres and their associated kinetochores [10]. In particular, the constitutive centromere associated network (CCAN) are required for proper spindle attachment, chromosome congression,

mitotic checkpoint activity and separation of sister chromatids during mitosis, leading to the assembly of a functional kinetochore [11]. The CCAN network is comprised of *CENP-A/B/C/F/I/J/M/O/P/Q/T/V/E/H/K/L/N/U/W* [12]. Previous studies showed that centromere-associated genes are detected in a variety of solid tumors and myeloma. In solid tumors, Zhang *et al.* demonstrated that centromere and kinetochore gene misexpression predicts cancer patient survival and response to radiotherapy and chemotherapy [13]; In MM, Kryukov *et al.* focused on centrosome-related genes (CAGP model: *CENP-A*, *CENP-E* and so on) that reveal the molecular heterogeneity characteristics and survival for MM patients [14, 15]. Nevertheless, in spite of advances in centromere, most other centromere-associated genes undefined abnormalities forming genetic complexity in MM may still exist, and none of these studies focused on MM risk stratification for all centromere-associated genes.

On these bases, we investigated centromere-associated gene signature able to distinguish the different stages of myeloma progression, and constructed a risk stratification model based on centromere-associated gene score (CGS) in MM. As a result, CGS was demonstrated to be an efficient model in prediction of clinical outcome, and enhanced our understanding of CIN in MM risk stratification.

Materials and methods

Gene expression profiling (GEP) and data analysis

Gene Expression Omnibus (GEO) database was carried out to measure the expressions of *CENP-E/H/K/L/N/U/W* in 2301 MM patients (GSE5900 [16], GSE2658 [4], GSE24080 [17], GSE31161 [18] and GSE9782 [19]). Data acquisition and normalization methods in above datasets have been described previously [17]. The expressions of *CENP-E/H/K/L/N/U/W* in plasma cells were determined using the Affymetrix U133Plus2.0 microarray (Affymetrix, USA), which was performed as previously described [4].

Statistical analysis

Various statistical analyses were utilized to assess the roles of *CENP-E/H/K/L/N/U/W* on clinical features and prognosis of MM patients. Two-tailed Student's t-test and One-way analysis of variance were adopted to compare two or multiple experimental groups. The Fisher's test was used to compare clinicopathological features between the high/low expressions of *CENP-E/H/K/L/N/U/W*. Survival curves were plotted according to the Kaplan-Meier method, and the log-rank test was employed to analyze statistical differences between survival curves. The effect of *CENP-E/H/K/L/N/U/W* on outcome was analyzed using univariate and multivariate Cox proportional hazard models. GraphPad Prism 6 software was employed for our analyses and $*P < 0.05$ was considered significant.

Results

CENP-E/H/K/L/N/U/W were high-risk myeloma genes

To evaluate the possibility that centromere-associated genes are crucial for myeloma, we examined centromere-associated gene expression in normal plasma (NP), smoldering multiple myeloma (SMM), monoclonal gammopathy of undetermined significance (MGUS) and myeloma cells using GEP database. Notably, *CENP-E/H/K/L/N/U/W* expressions increased significantly from NP, MGUS, SMM to MM samples (**Figure 1**, Supplementary Figure 1). In detail, we asked whether heightened *CENP-E/H/K/L/N/U/W* expressions might be related to a particular molecular subgroup of myeloma. **Figure 1** presented the *CENP-E/H/K/L/N/U/W* expressions in 8 widely recognized subgroups, showing that elevated *CENP-E/H/K/L/N/U/W* expressions are particularly prevalent in 3 known to confer high-risk in terms of clinical course and prognosis: MAF/MAFB (MF), MMSET/FGGR3 (MS) and Proliferation (PR). These findings led us to conclude that *CENP-E/H/K/L/N/U/W* are high-risk myeloma genes.

Correlations between CENP-E/H/K/L/N/U/W expressions and clinicopathological characteristics

To evaluate *CENP-E/H/K/L/N/U/W* expressions in MM patients, we divided MM patients into two categories according to their *CENP-E/H/K/L/N/U/W* expressions (low/high expression, using the 50th percentile as cut-offs). The clinicopathological characteristics according to

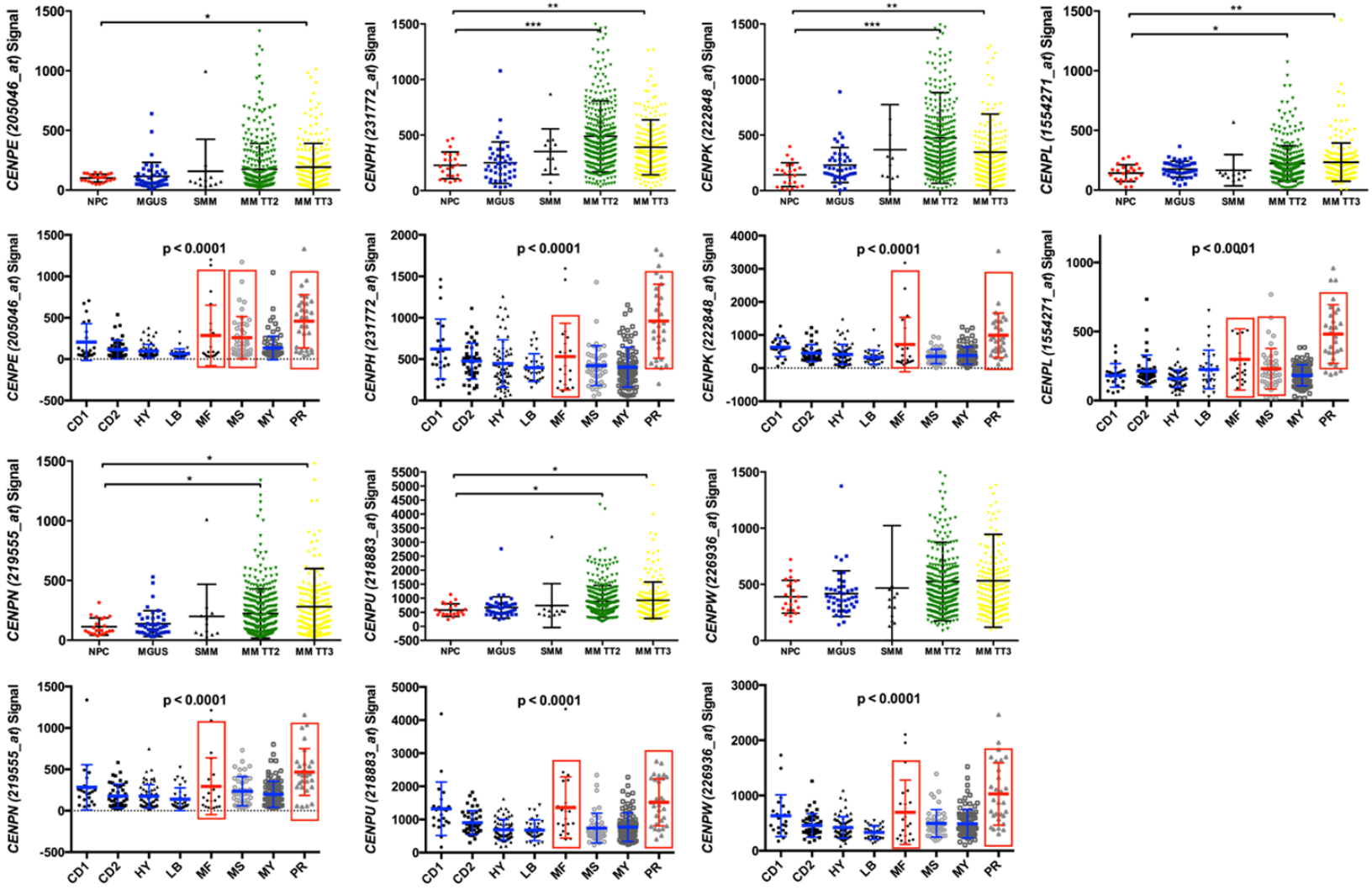


Figure 1. *CENP-E/H/K/L/N/U/W* were high-risk myeloma genes. (Upper row) *CENP-E/H/K/L/N/U/W* expressions of NP (n = 22), MGUS (n = 44), SMM (n = 12) and MM (n = 559) in GSE5900 and GSE2658 datasets. (Lower row) scatter-plots showed *CENP-E/H/K/L/N/U/W* expressions in eight MM subgroups (CD1 and CD2 subgroups with spiked expression of CCND1 and CCND3; PR, Proliferation; LB, Low-bone disease; HY, Hyperdiploid; MS, MMSET; MF, MAFB; MY, Myeloid) (*P < 0.05, **P < 0.01, ***P < 0.001).

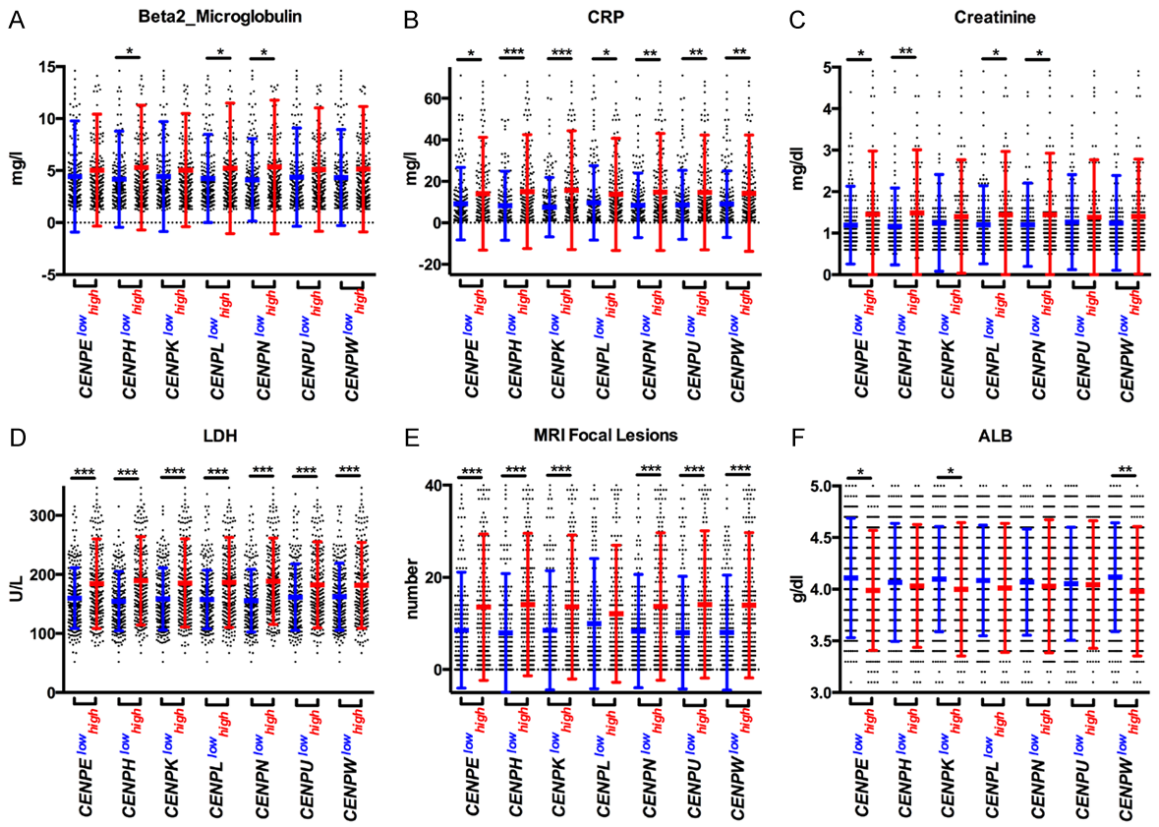


Figure 2. Correlations between *CENP-E/H/K/L/N/U/W* expressions and clinicopathological characteristics. A-E. High *CENP-E/H/K/L/N/U/W* expressions were significantly associated with high β2-MG, CRP, LDH, Creat and MRI focal lesions levels. F. High *CENP-E/H/K/L/N/U/W* expressions were significantly associated with low serum ALB level (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

CENP-E/H/K/L/N/U/W expressions were listed in Supplementary Tables 1, 2, 3, 4, 5, 6, 7. No significant correlations were observed between *CENP-E/H/K/L/N/U/W* expressions and other clinicopathological features such as sex, age, aspirate plasma cells (ASPC) and bone marrow biopsy plasma cells (BMPC). High *CENP-E/H/K/L/N/U/W* expressions were significantly associated with low serum albumin (ALB) and serum haemoglobin (HB) levels. On the contrary, high *CENP-E/H/K/L/N/U/W* expressions were also significantly associated with high β2-Microglobulin (β2-MG), C-reactive protein (CRP), creatinine (Creat), lactate dehydrogenase (LDH) and MRI focal lesions levels (**Figure 2** and Supplementary Tables 1, 2, 3, 4, 5, 6, 7).

CENP-E/H/K/L/N/U/W were linked to disease progression and relapse in MM

To validate our findings, we also evaluated the efficiency of centromere-associated genes in myeloma cell proliferation (**Figure 3A**). *CENP-E/N/U* expressions were positively correlated ($r = 0.6643$; $r = 0.6964$; $r = 0.6134$; $P < 0.0001$) with cell proliferation in 246 bortezomib-treated MM patients available at GSE9782, using the gene expression-based proliferation index (GPI) of myeloma devised by Mayo Clinic as proxy of effective tumor cell proliferation [20]. In addition, *CENP-E/H/K/L/N/U/W* expressions significantly increased in the relapsed MM patients from TT2 and TT3 cohorts compared to baseline patients in GSE31161 (**Figure 3B**). These data strongly suggested that *CENP-E/H/K/L/N/U/W* expressions could be adopted in the evolution of myeloma progression and relapse.

Increased CENP-E/H/K/L/N/U/W expressions correlated with poor prognosis in MM

To assess the survival time with *CENP-E/H/K/L/N/U/W* expressions in MM, we divided all MM patients into two groups based on high/

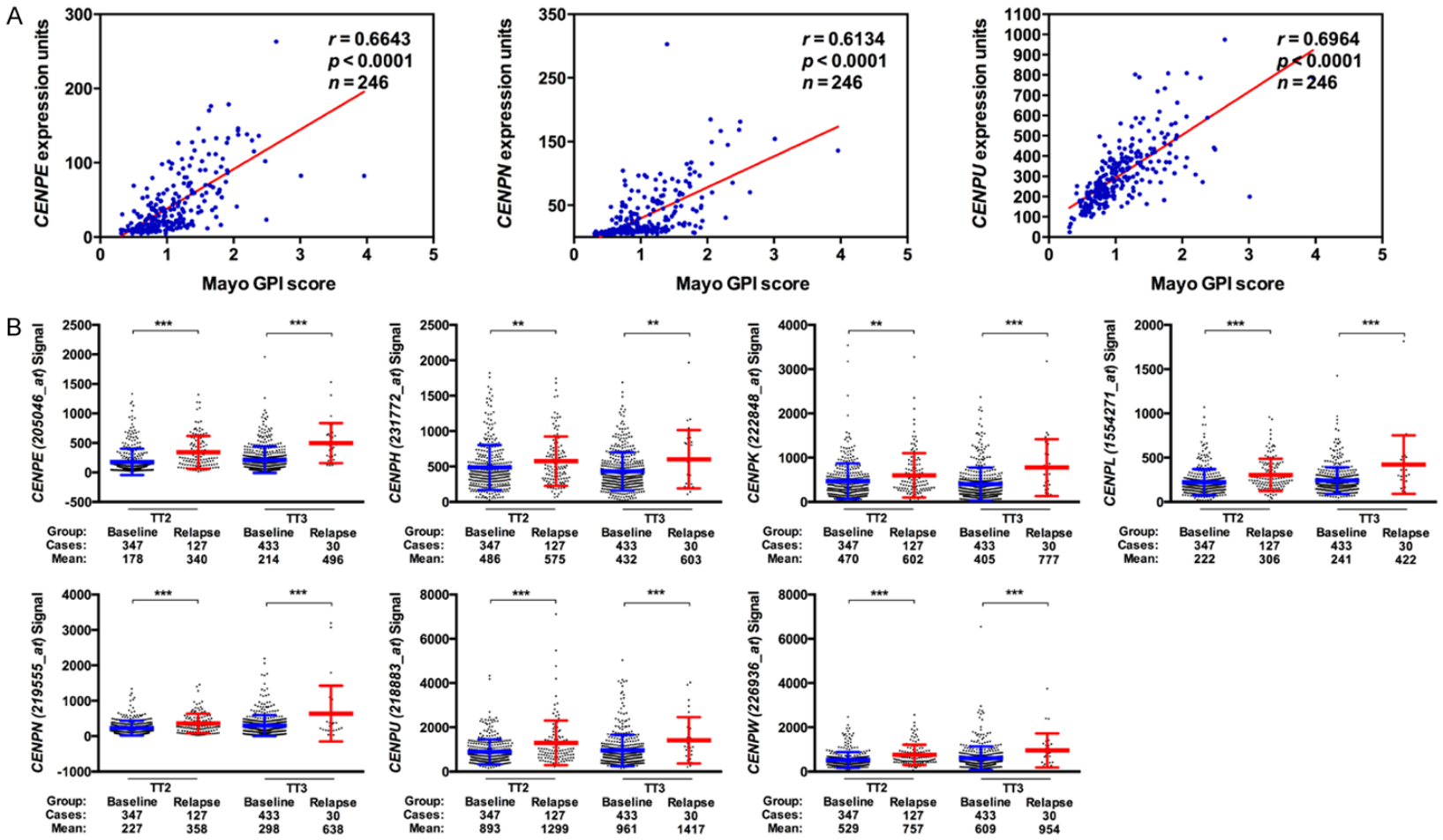


Figure 3. *CENP-E/H/K/L/N/U/W* is linked to disease progression and relapse in MM. A. Scatter plots demonstrating positive correlation of *CENP-E/H/K/L/N/U/W* expression and myeloma proliferation in 246 bortezomib-treated patients from the Mayo Clinic. Tumor cell proliferation was scored with the assistance of a gene expression-based proliferation index (GPI) developed by Bergsagel *et al.* B. The expressions of *CENP-E/H/K/L/N/U/W* were significantly up-regulated in relapsed patients from TT2 and TT3 cohort in comparison with baseline patients (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

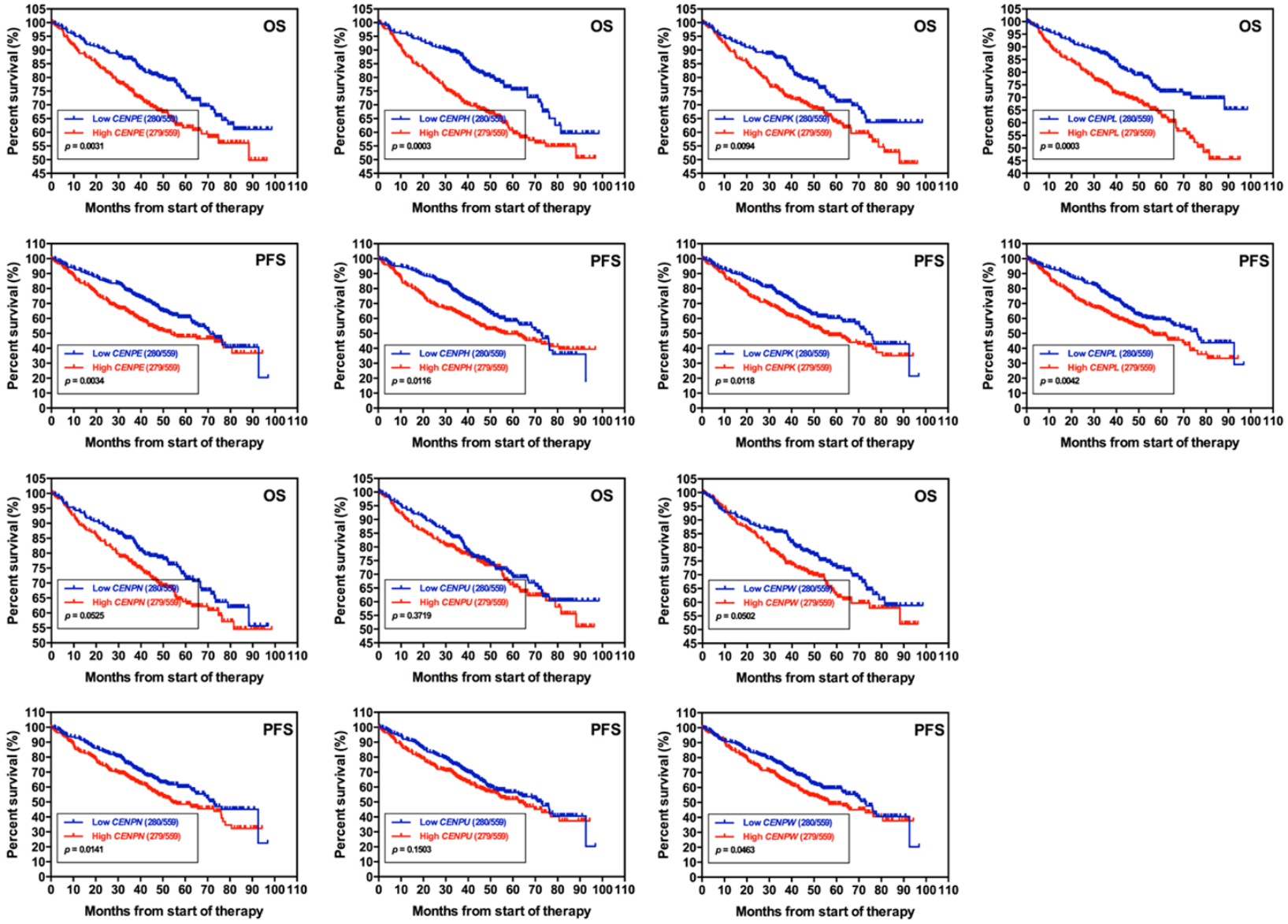


Figure 4. Increased *CENP-E/H/K/L/N/U/W* expressions correlated with poor prognosis in MM. Kaplan-Meier analyses showed OS (Upper row) and PFS (Lower row) of 559 newly diagnosed MM patients.

Table 1. Univariate Cox regression analyses for OS and PFS in 559 MM patients

Variables	OS			PFS		
	HR	95% CI	*p*	HR	95% CI	*p*
CENPE^{high}	1.590	1.174-2.153	0.003	1.450	1.137-1.876	0.004
CENPH^{high}	1.767	1.297-2.408	0.000	1.378	1.072-1.771	0.012
CENPK^{high}	1.499	1.105-2.035	0.009	1.377	1.071-1.770	0.013
CENPL^{high}	1.712	1.261-2.326	0.001	1.449	1.128-1.881	0.004
CENPN^{high}	1.348	0.998-1.822	0.052	1.362	1.081-1.749	0.015
CENPU^{high}	1.159	0.859-1.564	0.334	1.209	0.943-1.551	0.134
CENPW^{high}	1.355	1.003-1.832	0.048	1.292	1.007-1.658	0.044

low *CENP-E/H/K/L/N/U/W* expressions, The high *CENP-E/H/K/L/N/U/W* expression groups had shorter median OS and PFS time than low expression groups. As shown in **Figure 4**, MM patients with strong *CENP-E/H/K/L/N/U/W* expressions had an inferior OS and PFS. Additionally, we used the univariate cox analysis to evaluate *CENP-E/H/K/L/N/U/W* expressions on clinical outcomes, *CENP-E/H/K/L/N/U/W* resulted independently associated with survival (**Table 1**). To further understand the regulatory mechanisms of *CENP-E/H/K/L/N/U/W*, *CENP-E/H/K/L/N/U/W* associated with predicted targeted genes were analyzed using GEO database. It was identified that *CENP-E/H/K/L/N/U/W* expressions were highly correlated with transcription factor *FOXM1* expression ($r > 0.30$, $*P < 0.05$; **Figure 5A**, Supplementary Figure 2). To confirm this hypothesis, by combining *CENP-E/H/K/L/N/U/W* and *FOXM1*, we found that MM patients with high expression (cutoff: 50%, high *vs*. low) of *CENP-E/H/K/L/N/U/W* and *FOXM1* simultaneously had the worst prognosis compared to the patients with low expression of two genes together and the rest of the MM patients (medium) for both OS and PFS in GSE24080 (**Figure 5B**).

Construction of a centromere-associated gene score model

We added a score to *CENP-E/H/K/L/N/U/W* (high expression = 1 and low expression = 0) and then constructed centromere-associated gene score (CGS) model as follows: CENPE + CENPH + CENPK + CENPL + CENPN + CENPU + CENPW. The CGS model could assume 8 different values and according to 50th percentile, patients were divided into three groups: low-risk (LR) = CGS 0-1, intermediate-risk (IR) = CGS 2-5 and high-risk (HR) = CGS 6-7. Then, we calculated the CGS of each MM patient in GSE24080. All patients were divided into CGS^{LR} group (n = 150), CGS^{IR} group (n = 272) and CGS^{HR} group (n = 137) according to their risk fraction (**Figure 6A**). As a result, CGS model was strongly related to survival, with patients in CGS^{LR} group having better OS and PFS compared to CGS^{HR} group in GSE24080 (**Figure 6B** and **6C**). Additionally, we used the univariate and multivariate cox analysis to evaluate CGS and clinicopathological characteristics on clinical outcomes (**Tables 2** and **3**). The OS was decreased for CGS^{HR} group versus CGS^{LR+IR} groups (HR = 1.401, 95% CI: 1.008-1.948, P = 0.045), as well as PFS (HR = 1.379, 95% CI: 1.041-1.825, P = 0.025). To confirm the robustness of the CGS, we tested CGS model in predicted clinicopathological parameters distribution. Using 6 clinicopathological parameters, we identified different distribution among risk subgroups in 559 patients. Respectively, the levels of β2-MG, CRP, LDH, Creat and bone lesions were significantly increased in CGS^{HR} group compared to CGS^{LR} group. In contrast, ALB was obviously decreased in CGS^{HR} group (**Figure 6D**).

Evaluation of the CGS model in different datasets

We validated CGS model in other independent datasets, **Figure 7A** presented the values of CGS in 8 genetic subgroups of MM, showing that increased CGS is particularly distributed in high-risk subgroups. In sync with that, we observed a significantly increasing between the Zhan *et al.* defined two risk categories (low-risk groups: CD1 + CD2 + LB + HY + MY *vs.* high-risk groups: MF + MS + PR; 3.175 ± 0.014 *vs.* 4.351 ± 0.256, $P < 0.0001$). In addition, we utilized the Kaplan-Meier analysis to validated CGS model in two independent datasets, and the Kaplan-Meier survival analysis indicated that CGS^{LR} group had better OS compared to CGS^{HR} in TT2 (induction therapy: D(T)-PACE, Dex with or without thalidomide) and TT6 (autologous hematopoietic stem cell transplant) cohorts (**Figure 7B** and **7C**).

A

Correlation (Pearson r) *p < 0.05	CENPE	CENPH	CENPK	CENPL	CENPN	CENPU	CENPW
FOXM1	0.58*	0.40*	0.49*	0.49*	0.59*	0.62*	0.67*
FOXE1	-0.07*	-0.06	-0.07	0	-0.08	-0.03	0
CREB1	0.12*	0.01	-0.09*	0.18*	0.01	0.02	-0.04
E2F1	0.18*	0.09*	0.10*	0.12*	0.14*	0.15*	0.08*

B

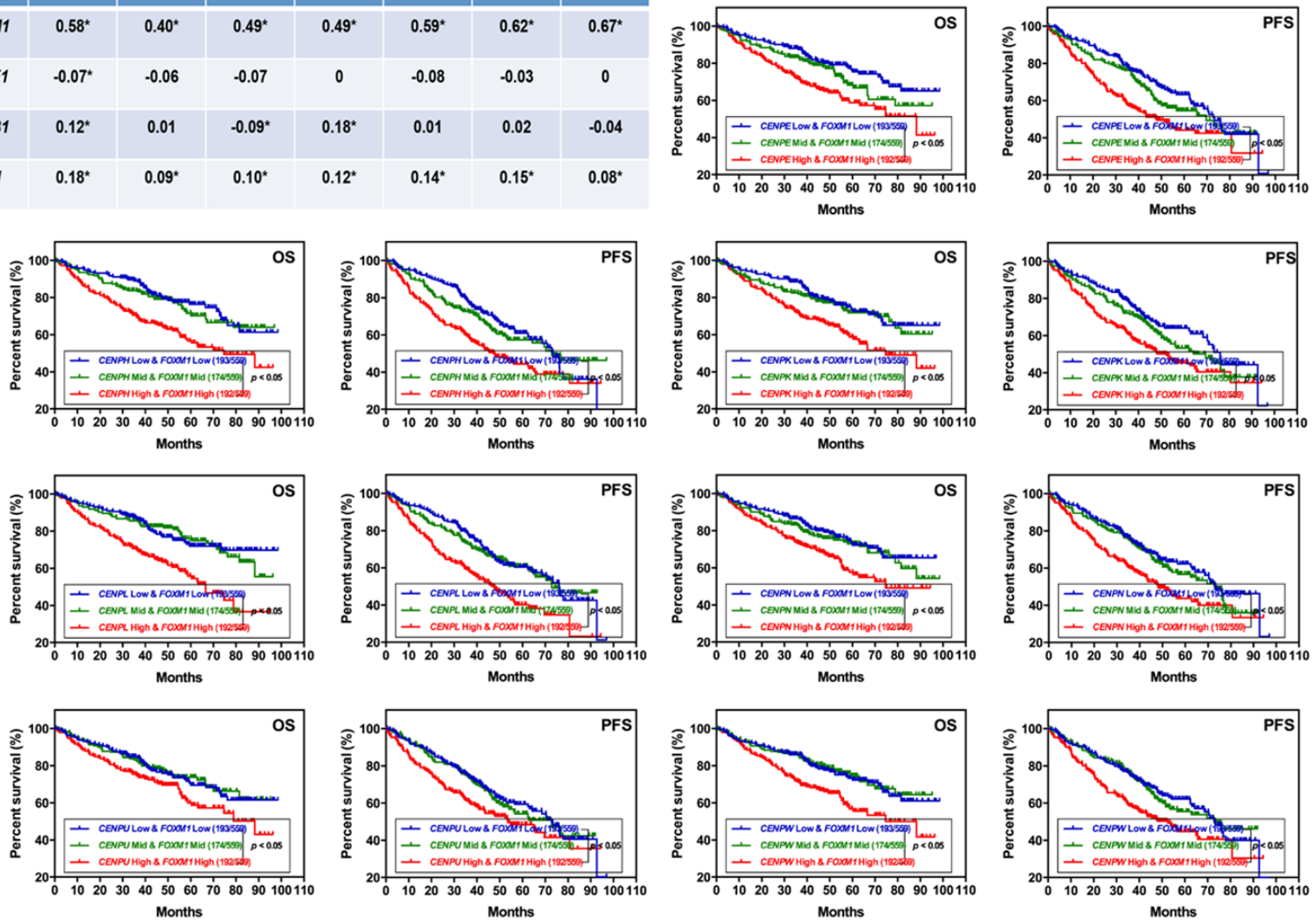


Figure 5. Relationship between the expressions of *CENP-E/H/K/L/N/U/W* and *FOXM1* in MM. A. Pearson's correlations between the transcript level patterns of *CENP-E/H/K/L/N/U/W* and predicted targeted genes (*$P < 0.05$). B. Survival analyses were performed based on the combination of *CENP-E/H/K/L/N/U/W* and *FOXM1* expressions. Kaplan-Meier showed OS and PFS curves of GSE24080.

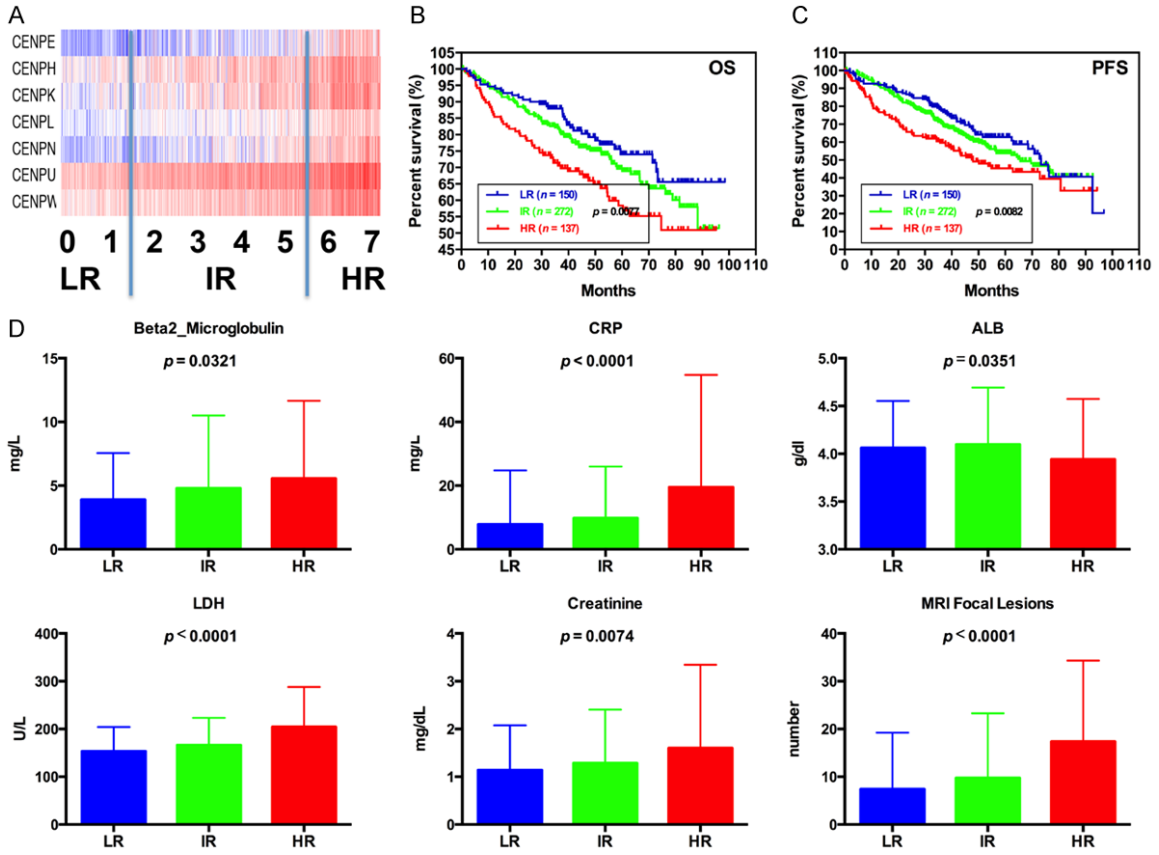


Figure 6. The correlations between CGS model and disease progression. A. Heat map (upper row) reporting probe fluorescence intensity of 7 selected genes for each patient evaluated in accordance with its survival, CGS risk score (lower row). B, C. The CGS^{HR} group identified MM patients with the lowest OS and EFS in GSE24080. D. The CGS^{HR} group was significantly associated with high β2-MG, CRP, LDH, Creat and MRI focal lesions levels. In contrast, CGS^{LR} group was significantly associated with high serum ALB level.

Table 2. Univariate and Multivariate Cox regression analyses for OS in 559 MM patients

Variables	Univariate model			Multivariate model		
	HR	95% CI	*p*	HR	95% CI	*p*
Age ≥ 65 yr	1.206	0.855-1.700	0.286			
Male sex	0.968	0.714-1.313	0.835			
β2-MG ≥ 3.5 mg/L	2.185	1.613-2.958	0.000	1.867	1.330-2.647	0.000
Creat ≥ 1.2 mg/dL	1.731	1.278-2.345	0.000	1.210	0.862-1.699	0.271
CRP ≥ 4 mg/L	1.539	1.132-2.092	0.006	1.353	0.985-1.859	0.062
ALB ≥ 3.5 g/dL	0.521	0.360-0.756	0.001	0.704	0.478-1.035	0.074
CGS^{HR}	1.583	1.147-2.185	0.005	1.401	1.008-1.948	0.045

Discussion

MM remains incurable despite novel treatments, and plenty of prognostic markers that reflect tumor- or host-related factors have failed to explain thoroughly the heterogeneity in clinical outcomes [21]. Therefore, it is important to stratify risk stratification for MM patients. With advances in MM study, several prognostic systems were constructed using previously reported prognostic parameters [22, 23]. However, these prognostic factors could not completely reflect the real prognostic condition of MM patients. Thus, evaluating a novel and powerful MM prognostic model is crucial for predicting the prognosis and determining personalized anti-MM treatment.

In the present study, *CENP-E/H/K/L/N/U/W* were significantly higher expressed in aggressive subgroups of myeloma (MF, MS and PR),

Table 3. Univariate and Multivariate Cox regression analyses for PFS in 559 MM patients

Variables	Univariate model			Multivariate model		
	HR	95% CI	*p*	HR	95% CI	*p*
Age ≥ 65 yr	1.138	0.853-1.518	0.379			
Male sex	0.990	0.768-1.275	0.936			
β2-MG ≥ 3.5 mg/L	1.903	1.482-2.445	0.000	1.773	1.329-2.364	0.000
Creat ≥ 1.2 mg/dL	1.469	1.136-1.889	0.003	1.087	0.813-1.455	0.573
CRP ≥ 4 mg/L	1.290	1.002-1.659	0.048	1.154	0.890-1.496	0.280
ALB ≥ 3.5 g/dL	0.665	0.477-0.927	0.016	0.827	0.586-1.165	0.277
CGS^{HR}	1.493	1.135-1.963	0.004	1.379	1.048-1.825	0.025

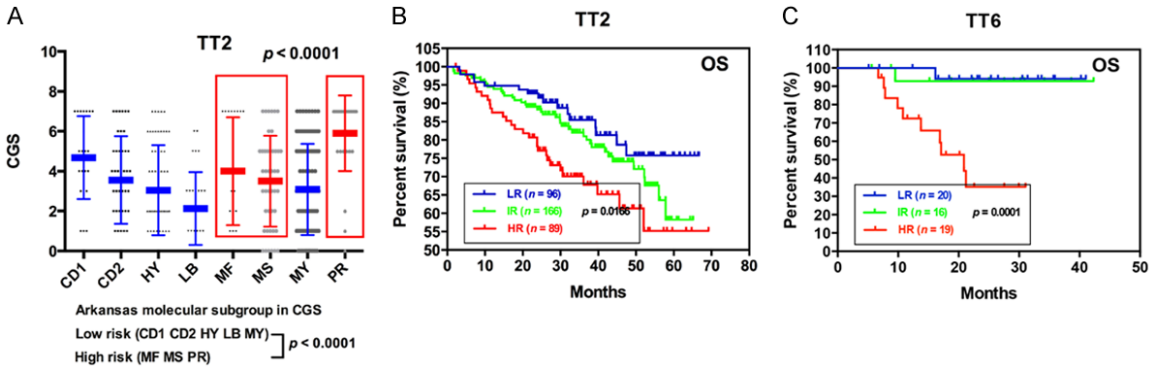


Figure 7. Validation of the CGS model in independent datasets. A. A scatter-plot showed CGS in eight MM subgroups. B. The CGS^{HR} group identified MM patients with the lowest OS in GSE2658. C. The CGS^{HR} group identified MM patients with the lowest OS in GSE57317.

which are characterized by high-risk MM and associated with an adverse prognosis [4, 24]. We also analyzed the prognostic significance of *CENP-E/H/K/L/N/U/W* in MM and correlated with markers of myeloma activity, such as lower levels of ALB, higher levels of β2-MG, Creat, LDH CRP and MRI focal lesions. Among them, International Staging System (ISS) has been constructed which combines biomarkers of tumor burden (ALB and β2-MG) with biomarkers of aggressive myeloma biology (bone lesions and LDH) [25, 26]. ALB and renal function have been considered easy and good indicators of survival [27]. The serum level of β2-MG is one of the most important independent predictors of survival and considered an indicator of tumor burden [28]. High levels of circulating LDH enhance myeloma cell proliferation and drug resistance under stressed conditions, and correlate with poor prognosis in myeloma [29-31]. Another interesting finding in this study is that the *CENP-E/H/K/L/N/U/W* expressions appear to correlate with response to dexamethasone or bortezomib-based chemotherapy. High-dose dexamethasone is commonly used for myeloma treatment [32]. Bortezomib, which targets the 26S proteasome subunit β5, has induced a high level of positive response rates [33, 34]. However, toxicities associated with global proteasomal inhibition and resistance to bortezomib or dexamethasone in MM are major concerns, prompting the further development of novel target and therapies. A great deal of variance was exhibited in *CENP-E/H/K/L/N/U/W*, and suggested new potential mechanisms of therapeutic molecules. More importantly, our results supported the fact that *CENP-E/H/K/L/N/U/W* might have prognostic values, and high expression groups had significantly shorter OS and PFS.

Following bioinformatics analysis, the present study identified that *CENP-E/H/K/L/N/U/W* had significant correlations with *FOXM1* expression, and *FOXM1* is also highly expressed in MM [35, 36]. In addition, the overall survival rate of patients with high expression of *FOXM1* was worse. However, there was no investigation between the survival trend of *FOXM1* and the survival trend of *CENP-E/H/K/L/N/U/W*. FO-

XM1 is a transcription factor that participates in all stages of biological functions, including cell proliferation and cell cycle, DNA damage repairs and cell self-renewal, which are involved in tumor progression and the response of chemotherapy [37, 38]. In regard to different biological functions attributable to FOXM1 in MM, the transcription factor seems to resemble well-established "master" transcription factors, such as IRF4 and MYC [39, 40]. Therefore, the present study hypothesized that CENP-E/H/K/L/N/U/W may be involved in FOXM1 regulatory network of MM (Supplementary Figure 2).

The above results provided stable support for the centromere-associated gene signature in the biologic function of myeloma cells. On these bases, we constructed a prognostic risk score with MM patients classified into three risk groups. Firstly, we analyzed the prognostic significance of CGS model in MM, CGS^{HR} group correlated with markers of myeloma activity, such as lower levels of ALB and HB, higher levels of LDH, CRP, bone lesions and β2-MG. More importantly, CGS^{HR} group correlated significantly to all the aforementioned parameters of disease activity, which support the fact that CGS model might have prognostic value. Next, the scatter plot showed that the CGS model was similar to 8-subgroup model among all groups. Aggressive subgroups of myeloma also had significantly higher CGS compared to all other molecular subgroups. At last, we analyzed the correlations between gene expression and clinical outcome based on CGS model in independent datasets. Our result showed that there was significant difference in the survival conditions of CGS^{HR}, CGS^{IR} and CGS^{LR} patients. Univariate and multivariate Cox proportional hazard regression analyses were then performed to verify the association of clinicopathological parameters and CGS model with survival. Our results further testified that the CGS^{HR} is an independent prognostic factor.

In conclusion, our results demonstrated the prognostic and predictive power of the CGS model, suggested a role for centromere misregulation in MM progression. Incorporation of CGS model into risk determination algorithms for newly-diagnosed MM patients will facilitate the development of CIN-targeted treatments.

Acknowledgements

This work was supported by the Jiangsu Provincial Medical Innovation Team (CXTDA2017-046).

Disclosure of conflict of interest

None.

Address correspondence to: Hua Bai, Department of Hematology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing 210008, China. E-mail: baihua92@126.com; Bing Chen, Department of Hematology, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, Nanjing 210008, China. E-mail: chenbing-2004@126.com

References

[1] Palumbo A and Anderson K. Multiple myeloma. N Engl J Med 2011; 364: 1046-1060.

[2] Bai H, Zhu H, Yan Q, Shen X, Lu X, Wang J, Li J and Chen L. TRPV2-induced Ca(2+)-calcineurin-NFAT signaling regulates differentiation of osteoclast in multiple myeloma. Cell Commun Signal 2018; 16: 68.

[3] Walker BA, Leone PE, Chiecchio L, Dickens NJ, Jenner MW, Boyd KD, Johnson DC, Gonzalez D, Dagrada GP, Protheroe RK, Konn ZJ, Stockley DM, Gregory WM, Davies FE, Ross FM and Morgan GJ. A compendium of myeloma-associated chromosomal copy number abnormalities and their prognostic value. Blood 2010; 116: e56-65.

[4] Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, Gupta S, Epstein J, Yaccoby S, Sawyer J, Burington B, Anaissie E, Hollmig K, Pineda-Roman M, Tricot G, van Rhee F, Walker R, Zangari M, Crowley J, Barlogie B and Shaughnessy JD Jr. The molecular classification of multiple myeloma. Blood 2006; 108: 2020-2028.

[5] Shaughnessy JD Jr, Zhan F, Burington BE, Huang Y, Colla S, Hanamura I, Stewart JP, Kordsmeier B, Randolph C, Williams DR, Xiao Y, Xu H, Epstein J, Anaissie E, Krishna SG, Cottler-Fox M, Hollmig K, Mohiuddin A, Pineda-Roman M, Tricot G, van Rhee F, Sawyer J, Alsayed Y, Walker R, Zangari M, Crowley J and Barlogie B. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood 2007; 109: 2276-2284.

[6] Decaux O, Lode L, Magrangeas F, Charbonnel C, Gouraud W, Jezequel P, Attal M, Harousseau JL, Moreau P, Bataille R, Campion L, Avet-Loiseau H and Minvielle S; Intergroupe Franco-

phone du Myélome. Prediction of survival in multiple myeloma based on gene expression profiles reveals cell cycle and chromosomal instability signatures in high-risk patients and hyperdiploid signatures in low-risk patients: a study of the Intergroupe Francophone du Myelome. J Clin Oncol 2008; 26: 4798-4805.

[7] Lee AJ, Endesfelder D, Rowan AJ, Walther A, Birkbak NJ, Futreal PA, Downward J, Szallasi Z, Tomlinson IP, Howell M, Kschischo M and Swanton C. Chromosomal instability confers intrinsic multidrug resistance. Cancer Res 2011; 71: 1858-1870.

[8] Wang W, Zhang Y, Chen R, Tian Z, Zhai Y, Janz S, Gu C and Yang Y. Chromosomal instability and acquired drug resistance in multiple myeloma. Oncotarget 2017; 8: 78234-78244.

[9] Burrell RA, McGranahan N, Bartek J and Swanton C. The causes and consequences of genetic heterogeneity in cancer evolution. Nature 2013; 501: 338-345.

[10] Cleveland DW, Mao Y and Sullivan KF. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. Cell 2003; 112: 407-421.

[11] Negrini S, Gorgoulis VG and Halazonetis TD. Genomic instability--an evolving hallmark of cancer. Nat Rev Mol Cell Biol 2010; 11: 220-228.

[12] Reinhold WC, Erliandri I, Liu H, Zoppoli G, Pommier Y and Larionov V. Identification of a predominant co-regulation among kinetochore genes, prospective regulatory elements, and association with genomic instability. PLoS One 2011; 6: e25991.

[13] Zhang W, Mao JH, Zhu W, Jain AK, Liu K, Brown JB and Karpen GH. Centromere and kinetochore gene misexpression predicts cancer patient survival and response to radiotherapy and chemotherapy. Nat Commun 2016; 7: 12619.

[14] Kryukov F, Nemec P, Dementyeva E, Kubiczkova L, Ihnatova I, Budinska E, Jarkovsky J, Sevcikova S, Kuglik P and Hajek R. Molecular heterogeneity and centrosome-associated genes in multiple myeloma. Leuk Lymphoma 2013; 54: 1982-1988.

[15] Kryukov F, Nemec P, Radova L, Kryukova E, Okubote S, Minarik J, Stefanikova Z, Pour L and Hajek R. Centrosome associated genes pattern for risk sub-stratification in multiple myeloma. J Transl Med 2016; 14: 150.

[16] Zhan F, Barlogie B, Arzoumanian V, Huang Y, Williams DR, Hollmig K, Pineda-Roman M, Tricot G, van Rhee F, Zangari M, Dhodapkar M and Shaughnessy JD Jr. Gene-expression signature of benign monoclonal gammopathy evident in multiple myeloma is linked to good prognosis. Blood 2007; 109: 1692-1700.

[17] Shi L, Campbell G, Jones WD, Campagne F, Wen Z, Walker SJ, Su Z, Chu TM, Goodsaid FM, Pusztai L, Shaughnessy JD Jr, Oberthuer A, Thomas RS, Paules RS, Fielden M, Barlogie B, Chen W, Du P, Fischer M, Furlanello C, Gallas BD, Ge X, Megherbi DB, Symmans WF, Wang MD, Zhang J, Bitter H, Brors B, Bushel PR, Bylesjo M, Chen M, Cheng J, Cheng J, Chou J, Davison TS, Delorenzi M, Deng Y, Devanarayan V, Dix DJ, Dopazo J, Dorff KC, Elloumi F, Fan J, Fan S, Fan X, Fang H, Gonzaludo N, Hess KR, Hong H, Huan J, Irizarry RA, Judson R, Juraeva D, Lababidi S, Lambert CG, Li L, Li Y, Li Z, Lin SM, Liu G, Lobenhofer EK, Luo J, Luo W, McCall MN, Nikolsky Y, Pennello GA, Perkins RG, Philip R, Popovici V, Price ND, Qian F, Scherer A, Shi T, Shi W, Sung J, Thierry-Mieg D, Thierry-Mieg J, Thodima V, Trygg J, Vishnuvajjala L, Wang SJ, Wu J, Wu Y, Xie Q, Yousef WA, Zhang L, Zhang X, Zhong S, Zhou Y, Zhu S, Arasappan D, Bao W, Lucas AB, Berthold F, Brennan RJ, Buness A, Catalano JG, Chang C, Chen R, Cheng Y, Cui J, Czika W, Demichelis F, Deng X, Dosymbekov D, Eils R, Feng Y, Fostel J, Fulmer-Smentek S, Fuscoe JC, Gatto L, Ge W, Goldstein DR, Guo L, Halbert DN, Han J, Harris SC, Hatzis C, Herman D, Huang J, Jensen RV, Jiang R, Johnson CD, Jurman G, Kahlert Y, Khuder SA, Kohl M, Li J, Li L, Li M, Li QZ, Li S, Li Z, Liu J, Liu Y, Liu Z, Meng L, Madera M, Martinez-Murillo F, Medina I, Meehan J, Miclaus K, Moffitt RA, Montaner D, Mukherjee P, Mulligan GJ, Neville P, Nikolskaya T, Ning B, Page GP, Parker J, Parry RM, Peng X, Peterson RL, Phan JH, Quanz B, Ren Y, Riccadonna S, Roter AH, Samuelson FW, Schumacher MM, Shambaugh JD, Shi Q, Shippy R, Si S, Smalter A, Sotiriou C, Soukup M, Staedtler F, Steiner G, Stokes TH, Sun Q, Tan PY, Tang R, Tezak Z, Thorn B, Tsyganova M, Turpaz Y, Vega SC, Visintainer R, von Frese J, Wang C, Wang E, Wang J, Wang W, Westermann F, Willey JC, Woods M, Wu S, Xiao N, Xu J, Xu L, Yang L, Zeng X, Zhang J, Zhang L, Zhang M, Zhao C, Puri RK, Scherf U, Tong W and Wolfinger RD; MAQC Consortium. The MicroArray Quality Control (MAQC)-II study of common practices for the development and validation of microarray-based predictive models. Nat Biotechnol 2010; 28: 827-838.

[18] Mitchell JS, Li N, Weinhold N, Forsti A, Ali M, van Duin M, Thorleifsson G, Johnson DC, Chen B, Halvarsson BM, Gudbjartsson DF, Kuiper R, Stephens OW, Bertsch U, Broderick P, Campo C, Einsele H, Gregory WA, Gullberg U, Henrion M, Hillengass J, Hoffmann P, Jackson GH, Johnsson E, Joud M, Kristinsson SY, Lenhoff S, Lenive O, Mellqvist UH, Migliorini G, Nahi H, Nelander S, Nickel J, Nothen MM, Rafnar T, Ross FM, da Silva Filho MI, Swaminathan B,

Thomsen H, Turesson I, Vangsted A, Vogel U, Waage A, Walker BA, Wihlborg AK, Broyl A, Davies FE, Thorsteinsdottir U, Langer C, Hansson M, Kaiser M, Sonneveld P, Stefansson K, Morgan GJ, Goldschmidt H, Hemminki K, Nilsson B and Houlston RS. Genome-wide association study identifies multiple susceptibility loci for multiple myeloma. Nat Commun 2016; 7: 12050.

[19] Mulligan G, Mitsiades C, Bryant B, Zhan F, Chng WJ, Roels S, Koenig E, Fergus A, Huang Y, Richardson P, Trepicchio WL, Broyl A, Sonneveld P, Shaughnessy JD Jr, Bergsagel PL, Schenkein D, Esseltine DL, Boral A and Anderson KC. Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib. Blood 2007; 109: 3177-3188.

[20] Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B and Shaughnessy J Jr. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. Blood 2005; 106: 296-303.

[21] Chng WJ, Dispenzieri A, Chim CS, Fonseca R, Goldschmidt H, Lentzsch S, Munshi N, Palumbo A, Miguel JS, Sonneveld P, Cavo M, Usmani S, Durie BG and Avet-Loiseau H; International Myeloma Working Group. IMWG consensus on risk stratification in multiple myeloma. Leukemia 2014; 28: 269-277.

[22] Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, Richardson P, Caltagirone S, Lahuerta JJ, Facon T, Bringhen S, Gay F, Attal M, Passera R, Spencer A, Offidani M, Kumar S, Musto P, Lonial S, Petrucci MT, Orlowski RZ, Zamagni E, Morgan G, Dimopoulos MA, Durie BG, Anderson KC, Sonneveld P, San Miguel J, Cavo M, Rajkumar SV and Moreau P. Revised international staging system for multiple myeloma: a report from international myeloma working group. J Clin Oncol 2015; 33: 2863-2869.

[23] Barlogie B, Bolejack V, Schell M and Crowley J. Prognostic factor analyses of myeloma survival with intergroup trial S9321 (INT 0141): examining whether different variables govern different time segments of survival. Ann Hematol 2011; 90: 423-428.

[24] van Andel H, Kocemba KA, de Haan-Kramer A, Mellink CH, Piwowar M, Broijl A, van Duin M, Sonneveld P, Maurice MM, Kersten MJ, Spaargaren M and Pals ST. Loss of CYLD expression unleashes Wnt signaling in multiple myeloma and is associated with aggressive disease. Oncogene 2017; 36: 2105-2115.

[25] Rajkumar SV. Myeloma today: disease definitions and treatment advances. Am J Hematol 2016; 91: 90-100.

[26] Greipp PR, San Miguel J, Durie BG, Crowley JJ, Barlogie B, Blade J, Boccadoro M, Child JA, Avet-Loiseau H, Kyle RA, Lahuerta JJ, Ludwig H, Morgan G, Powles R, Shimizu K, Shustik C, Sonneveld P, Tosi P, Turesson I and Westin J. International staging system for multiple myeloma. J Clin Oncol 2005; 23: 3412-3420.

[27] Jacobson JL, Hussein MA, Barlogie B, Durie BG and Crowley JJ; Southwest Oncology Group. A new staging system for multiple myeloma patients based on the Southwest Oncology Group (SWOG) experience. Br J Haematol 2003; 122: 441-450.

[28] Min R, Li Z, Epstein J, Barlogie B and Yi Q. Beta(2)-microglobulin as a negative growth regulator of myeloma cells. Br J Haematol 2002; 118: 495-505.

[29] Yang J, Liu Z, Liu H, He J, Yang J, Lin P, Wang Q, Du J, Ma W, Yin Z, Davis E, Orlowski RZ, Hou J and Yi Q. C-reactive protein promotes bone destruction in human myeloma through the CD32-p38 MAPK-Twist axis. Sci Signal 2017; 10: eaan6282.

[30] Ludwig H, Durie BG, Bolejack V, Turesson I, Kyle RA, Blade J, Fonseca R, Dimopoulos M, Shimizu K, San Miguel J, Westin J, Harousseau JL, Beksac M, Boccadoro M, Palumbo A, Barlogie B, Shustik C, Cavo M, Greipp PR, Joshua D, Attal M, Sonneveld P and Crowley J. Myeloma in patients younger than age 50 years presents with more favorable features and shows better survival: an analysis of 10 549 patients from the International Myeloma Working Group. Blood 2008; 111: 4039-4047.

[31] Dimopoulos MA, Barlogie B, Smith TL and Alexanian R. High serum lactate dehydrogenase level as a marker for drug resistance and short survival in multiple myeloma. Ann Intern Med 1991; 115: 931-935.

[32] Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T, Harousseau JL, Ben-Yehuda D, Lonial S, Goldschmidt H, Reece D, San-Miguel JF, Bladé J, Boccadoro M, Cavenagh J, Dalton WS, Boral AL, Esseltine DL, Porter JB, Schenkein D and Anderson KC; Assessment of Proteasome Inhibition for Extending Remissions (APEX) Investigators. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med 2005; 352: 2487-2498.

[33] Oerlemans R, Franke NE, Assaraf YG, Cloos J, van Zantwijk I, Berkers CR, Scheffer GL, Debipersad K, Vojtekova K, Lemos C, van der Heijden JW, Ylstra B, Peters GJ, Kaspers GL, Dijkmans BA, Scheper RJ and Jansen G. Molecular basis of bortezomib resistance: proteasome subunit beta5 (PSMB5) gene mutation and overexpression of PSMB5 protein. Blood 2008; 112: 2489-2499.

[34] Franke NE, Niewerth D, Assaraf YG, van Meerloo J, Vojtekova K, van Zantwijk CH, Zweegman S, Chan ET, Kirk CJ, Geerke DP, Schimmer AD,

Kaspers GJ, Jansen G and Cloos J. Impaired bortezomib binding to mutant beta5 subunit of the proteasome is the underlying basis for bortezomib resistance in leukemia cells. Leukemia 2012; 26: 757-768.

[35] Gu C, Holman C, Sompallae R, Jing X, Tomasson M, Hose D, Seckinger A, Zhan F, Tricot G, Goldschmidt H, Yang Y and Janz S. Upregulation of FOXM1 in a subset of relapsed myeloma results in poor outcome. Blood Cancer J 2018; 8: 22.

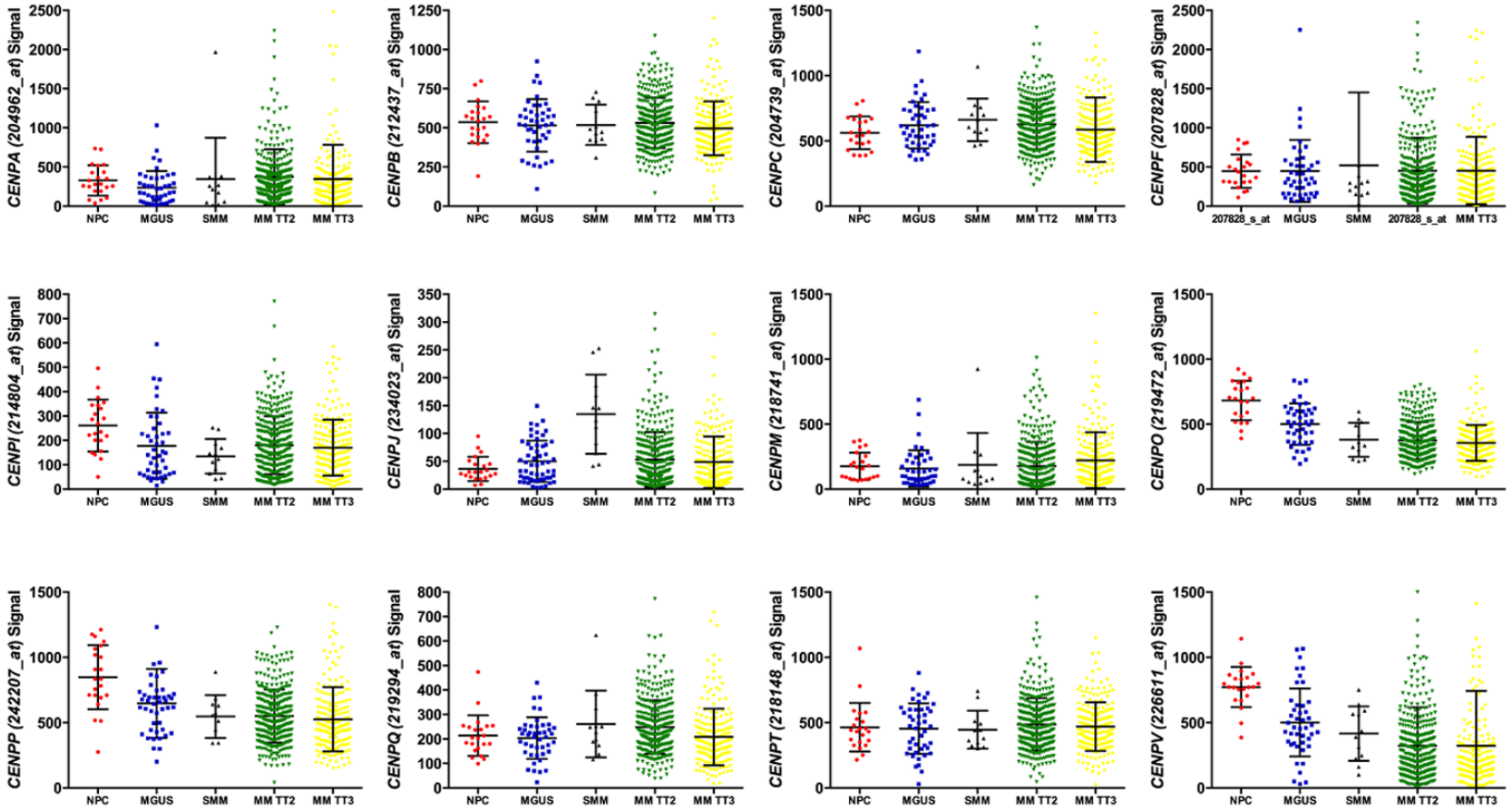
[36] Gu C, Yang Y, Sompallae R, Xu H, Tompkins VS, Holman C, Hose D, Goldschmidt H, Tricot G, Zhan F and Janz S. FOXM1 is a therapeutic target for high-risk multiple myeloma. Leukemia 2016; 30: 873-882.

[37] Bai H and Chen B. BAG3 regulates multiple myeloma cell proliferation through FOXM1/Rb/E2F axis. Cancer Gene Ther 2020; 27: 108-111.

[38] Zona S, Bella L, Burton MJ, Nestal de Moraes G and Lam EW. FOXM1: an emerging master regulator of DNA damage response and genotoxic agent resistance. Biochim Biophys Acta 2014; 1839: 1316-1322.

[39] Bai H, Wu S, Wang R, Xu J and Chen L. Bone marrow IRF4 level in multiple myeloma: an indicator of peripheral blood Th17 and disease. Oncotarget 2017; 8: 85392-85400.

[40] Keats JJ, Chesi M, Egan JB, Garbitt VM, Palmer SE, Braggio E, Van Wier S, Blackburn PR, Baker AS, Dispenzieri A, Kumar S, Rajkumar SV, Carpten JD, Barrett M, Fonseca R, Stewart AK and Bergsagel PL. Clonal competition with alternating dominance in multiple myeloma. Blood 2012; 120: 1067-1076.



Supplementary Figure 1. *CENP-A/B/C/F/I/J/M/O/P/T/V* expressions of NP (n = 22), MGUS (n = 44), SMM (n = 12) and MM (n = 599) in GSE5900 and GSE2658 dataset.

Supplementary Table 1. Correlations between *CENPE* expression and clinicopathological characteristics in 559 MM patients

Characteristic	*CENPE*^{low}	*CENPE*^{high}	*p* value
Age ≥ 65 yr	71/280 (25)	65/279 (23)	0.570†
Male sex	166/280 (59)	171/279 (61)	0.628†
β2-MG ≥ 3.5 (mg/L)	110/280 (39)	129/279 (46)	0.096†
CRP ≥ 4 (mg/L)	129/280 (46)	163/279 (58)	0.004*
Creat ≥ 1.2 (mg/dL)	80/280 (28)	102/279 (36)	0.047*
LDH ≥ 170 (U/L)	100/280 (35)	130/279 (46)	0.009*
ALB ≥ 3.5 (g/dL)	251/280 (89)	231/279 (82)	0.020*
HB ≥ 11 (g/dL)	168/280 (60)	144/279 (51)	0.050*
ASPC ≥ 40%	143/280 (51)	140/279 (50)	0.865*
BMPC ≥ 50%	138/280 (49)	127/279 (45)	0.397*
MRI ≥ 3 lesions	132/280 (47)	173/279 (62)	0.000*

*Fishers exact test was used. †The chi-square testwas used.

Supplementary Table 2. Correlations between *CENPH* expression and clinicopathological characteristics in 559 MM patients

Characteristic	*CENPH*^{low}	*CENPH*^{high}	*p* value
Age ≥ 65 yr	77/280 (27)	59/279 (21)	0.080†
Male sex	171/280 (61)	166/279 (59)	0.703†
β2-MG ≥ 3.5 (mg/L)	110/280 (39)	129/279 (46)	0.096†
CRP ≥ 4 (mg/L)	127/280 (45)	165/279 (59)	0.001*
Creat ≥ 1.2 (mg/dL)	72/280 (25)	110/279 (39)	0.000*
LDH ≥ 170 (U/L)	87/280 (31)	143/279 (51)	0.000*
ALB ≥ 3.5 (g/dL)	249/280 (88)	233/279 (85)	0.066*
HB ≥ 11 (g/dL)	163/280 (58)	149/279 (53)	0.268*
ASPC ≥ 40%	140/280 (50)	143/279 (51)	0.799*
BMPC ≥ 50%	135/280 (48)	130/279 (46)	0.735*
MRI ≥ 3 lesions	127/280 (45)	178/279 (63)	0.000*

*Fishers exact test was used. †The chi-square testwas used.

Supplementary Table 3. Correlations between *CENPK* expression and clinicopathological characteristics in 559 MM patients

Characteristic	*CENPK*^{low}	*CENPK*^{high}	*p* value
Age ≥ 65 yr	77/280 (27)	59/279 (21)	0.080†
Male sex	164/280 (58)	173/279 (62)	0.406†
β2-MG ≥ 3.5 (mg/L)	110/280 (39)	129/279 (46)	0.096†
CRP ≥ 4 (mg/L)	127/280 (45)	165/279 (59)	0.001*
Creat ≥ 1.2 (mg/dL)	79/280 (28)	103/279 (36)	0.030*
LDH ≥ 170 (U/L)	93/280 (33)	137/279 (49)	0.000*
ALB ≥ 3.5 (g/dL)	248/280 (88)	234/279 (83)	0.112*
HB ≥ 11 (g/dL)	166/280 (59)	146/279 (52)	0.105*
ASPC ≥ 40%	144/280 (51)	139/279 (49)	0.735*
BMPC ≥ 50%	134/280 (47)	131/279 (46)	0.865*
MRI ≥ 3 lesions	130/280 (46)	175/279 (62)	0.004*

*Fishers exact test was used. †The chi-square testwas used.

Supplementary Table 4. Correlations between *CENPL* expression and clinicopathological characteristics in 559 MM patients

Characteristic	*CENPL*^{low}	*CENPL*^{high}	*p* value
Age ≥ 65 yr	70/280 (25)	66/279 (23)	0.137†
Male sex	177/280 (63)	160/279 (57)	0.156†
β2-MG ≥ 3.5 (mg/L)	111/280 (39)	128/279 (45)	0.136†
CRP ≥ 4 (mg/L)	132/280 (47)	160/279 (57)	0.017*
Creat ≥ 1.2 (mg/dL)	84/280 (30)	98/279 (35)	0.207*
LDH ≥ 170 (U/L)	94/280 (33)	136/279 (48)	0.000*
ALB ≥ 3.5 (g/dL)	243/280 (86)	239/279 (85)	0.714*
HB ≥ 11 (g/dL)	171/280 (61)	141/279 (50)	0.013*
ASPC ≥ 40%	146/280 (52)	137/279 (49)	0.499*
BMPC ≥ 50%	126/280 (45)	139/279 (49)	0.597*
MRI ≥ 3 lesions	142/280 (50)	163/279 (58)	0.074*

*Fishers exact test was used. †The chi-square testwas used.

Supplementary Table 5. Correlations between *CENPN* expression and clinicopathological characteristics in 559 MM patients

Characteristic	*CENPN*^{low}	*CENPN*^{high}	*p* value
Age ≥ 65 yr	68/280 (24)	68/279 (24)	0.980†
Male sex	165/280 (58)	172/279 (61)	0.511†
β2-MG ≥ 3.5 (mg/L)	109/280 (38)	130/279 (46)	0.072†
CRP ≥ 4 (mg/L)	131/280 (46)	161/279 (57)	0.011*
Creat ≥ 1.2 (mg/dL)	83/280 (29)	99/279 (35)	0.149*
LDH ≥ 170 (U/L)	86/280 (30)	144/279 (51)	0.000*
ALB ≥ 3.5 (g/dL)	247/280 (88)	235/279 (84)	0.179*
HB ≥ 11 (g/dL)	174/280 (62)	138/279 (49)	0.002*
ASPC ≥ 40%	143/280 (51)	140/279 (50)	0.865*
BMPC ≥ 50%	141/280 (50)	124/279 (44)	0.175*
MRI ≥ 3 lesions	139/280 (49)	166/279 (59)	0.021*

*Fishers exact test was used. †The chi-square testwas used.

Supplementary Table 6. Correlations between *CENPU* expression and clinicopathological characteristics in 559 MM patients

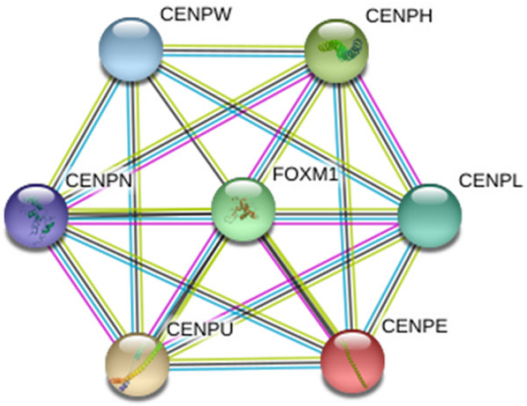
Characteristic	*CENPU*^{low}	*CENPU*^{high}	*p* value
Age ≥ 65 yr	71/280 (25)	65/279 (23)	0.570†
Male sex	168/280 (60)	169/279 (60)	0.889†
β2-MG ≥ 3.5 (mg/L)	110/280 (39)	129/279 (46)	0.096†
CRP ≥ 4 (mg/L)	134/280 (47)	158/279 (56)	0.042*
Creat ≥ 1.2 (mg/dL)	88/280 (31)	94/279 (33)	0.588*
LDH ≥ 170 (U/L)	103/280 (36)	127/279 (45)	0.039*
ALB ≥ 3.5 (g/dL)	238/280 (85)	244/279 (87)	0.461*
HB ≥ 11 (g/dL)	165/280 (58)	147/279 (52)	0.148*
ASPC ≥ 40%	142/280 (50)	141/279 (50)	1.000*
BMPC ≥ 50%	131/280 (46)	134/279 (48)	0.799*
MRI ≥ 3 lesions	132/280 (47)	173/279 (62)	0.000*

*Fishers exact test was used. †The chi-square testwas used.

Supplementary Table 7. Correlations between *CENPW* expression and clinicopathological characteristics in 559 MM patients

Characteristic	*CENPW*^{low}	*CENPW*^{high}	*p* value
Age ≥ 65 yr	75/280 (26)	61/279 (21)	0.263†
Male sex	162/280 (57)	175/279 (62)	0.239†
β2-MG ≥ 3.5 (mg/L)	112/280 (40)	127/279 (45)	0.187†
CRP ≥ 4 (mg/L)	137/280 (48)	155/279 (55)	0.127*
Creat ≥ 1.2 (mg/dL)	84/280 (30)	98/279 (35)	0.207*
LDH ≥ 170 (U/L)	103/280 (36)	127/279 (45)	0.039*
ALB ≥ 3.5 (g/dL)	248/280 (88)	234/279 (83)	0.112*
HB ≥ 11 (g/dL)	164/280 (58)	148/279 (53)	0.201*
ASPC ≥ 40%	152/280 (54)	131/279 (46)	0.090*
BMPC ≥ 50%	142/280 (50)	123/279 (44)	0.127*
MRI ≥ 3 lesions	124/280 (44)	181/279 (64)	0.000*

Abbrevations: Creat, Serum creatinine; CRP, C-reactive protein; ALB, Serum Albumin; β2-MG, β2-Microglobulin; LDH, Lactate Dehydrogenase; HB, Haemoglobin; ASPC, Aspirate plasma cells; BMPC, Bone marrow biopsy plasma cells; *Fisher's exact test was used. †The chi-square test was used.



Supplementary Figure 2. The protein network was constructed by online STRING software.