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ARTICLE

High expression of chaperonin-containing TCP1 subunit 3 may induce dismal prognosis in multiple myeloma

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Abstract

The prognosis role of *CCT3* in MM and the possible pathways it involved were studied in our research. By analyzing ten independent datasets (including 48 healthy donors, 2220 MM, 73 MGUS, and 6 PCL), *CCT3* was found to express higher in MM than healthy donors, and the expression level was gradually increased from MGUS, SMM, MM to PCL (all $P < 0.01$). By analyzing three independent datasets (GSE24080, GSE2658, and GSE4204), we found that *CCT3* was a significant indicator of poor prognosis (all $P < 0.01$). KEGG and GSEA analysis showed that *CCT3* expression was associated with JAK-STAT3 pathway, Hippo signaling pathway, and WNT signaling pathway. In addition, different expressed genes analysis revealed MYC, which was one of the downstream genes regulated by JAK-STAT3 pathway, was upregulated in MM. This confirms that JAK-STAT3 signaling pathway may promote the progress of disease which was regulated by *CCT3* expression. Our study revealed that *CCT3* may play a supporting role at the diagnosis of myeloid, and high expression of *CCT3* suggested poor prognosis in MM. *CCT3* expression may promote the progression of MM mainly by regulating *MYC* through JAK-STAT3 signaling pathway.

Background

Multiple myeloma (MM) is a malignant plasma cell disease, with clinical manifestations of anemia, bone pain, renal insufficiency and hemorrhage [1]. Some molecular have been conferred to be related with adverse prognosis of MM, such as RB1 and p53 related protein kinase [2, 3],

but more biomarkers are still needed at present. The expressions of some molecular biomarkers are closely related to the prognosis of MM, for example, high expression of *BCAR3* can predict a good prognosis in MM patients [4], overexpression of UBE2T and JAM-A predict poor prognosis [5, 6], and low serum miR-19a expression was a poor prognostic indicator in MM [7].

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Here we investigated the prognosis effect of *CCT3* expression on MM.

Chaperonin-containing T-complex protein1 (CCT) is a molecular chaperone protein, which plays a central role in assisting the folding of actin and tubulin to enhance cell migration [8, 9]. Cancer cell migration into surrounding tissues is the first step in cancer metastasis [10]. CCT has eight subunits, namely *CCT1-CCT8*, and they are irregulated in different types of cancers, such as CCT8 can promote the migration and invasion of esophageal squamous carcinoma by regulating actin and tubulin, CCT6A is a potential prognostic biomarker in glioblastoma [11]. *CCT3* is one of the subunits of CCT and is widely studied in different cancers. Higher level of *CCT3* expression in papillary thyroid carcinoma (PTC), and *CCT3* knockdown can inhibit the proliferation of PTC cells, affect cell cycle progression and promote apoptosis [12]. *CCT3* is overexpressed in gastric cancer, and *CCT3* knockdown can also suppresses the proliferation and induce cell apoptosis in gastric cancer [13].

Here we analyzed 2220 MM patients (2380 samples) to find out the prognosis effect of *CCT3* expression on MM and its possible action pathways. All the data were collected from ten independent datasets. In our study, we found that high *CCT3* expression is a dismal prognosis factor in MM patients, and its function intensity was positively correlated with the disease progression. In addition, *CCT3* may mainly influence the JAK-STAT3 signaling pathway to affect the progression of MM.

Methods

Patient samples

In our study, ten independent datasets (including GSE39754 [14–16], GSE5900 [17, 18], GSE2113 [17], GSE6477 [19, 20], GSE16558 [21], GSE82307 [21], GSE38627 [22], GSE24080 [23–25], GSE2658 [17, 25–31] and GSE4204 [18]) were enrolled, and they are derived from Gene Expression Omnibus database (GEO). All the datasets include 48 healthy donors, 2220 MM patients, 73 MGUS and 6 PCL (totally 2380 samples). The expressions of *CCT3* in healthy donors, MGUS patients, smoldering MM (SMM) patients, MM patients, relapsed MM (RMM) patients and PCL patients were analyzed (GSE39754, GSE5900, GSE2113, GSE6477). The differences of *CCT3* expression between different cytogenetic subtypes and normal donors (GSE16558), between new patients and relapsed patients (GSE82307), between patients with beta-2 microglobulin (B2M) ≥ 3.5 mg/L and B2M < 3.5 mg/L (GSE82307), and between patients with lactate dehydrogenase (LDH) ≥ 250 U/L and LDH < 250 U/L (GSE38627) were analyzed. And

the survivals between patients with high *CCT3* and low *CCT3* expressions were analyzed using GSE24080, GSE2658 and GSE4204 datasets.

All experiments design, quality control, and data normalization were in line with the standard Affymetrix protocols. All clinical, cytogenetic and molecular information as well as microarray data of these patients were publicly accessible at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo). The research was conducted in accordance International Conference on and the Declaration of Helsinki.

Survival analysis

Overall survival (OS) was defined as the time from the date of diagnosis to death due to any cause. Event-free survival (EFS) defined from date of registration to the occurrence of death from any cause, disease progression or relapse, or censored at the date of last contact. Patients from datasets of GSE24080 ($n = 559$), GSE2658 ($n = 559$) and GSE4204 ($n = 538$) were divided into two groups according to *CCT3* expression, separately. The EFS and OS were analyzed in GSE24080 dataset, and OS was analyzed in GSE2658 and GSE4204 datasets using Kaplan–Meier methods.

Statistical analysis

Clinical and molecular characteristics of MM patients from the GSE24080 ($n = 559$) were summarized using descriptive statistics. Data sets were described with median and/or percentage. Age data were compared using Cruskal–Wallis test, categorical data were compared using Chi-square test, and numerical data were compared using Welch Two Sample *t*-test. Multivariate Cox proportional hazard models were constructed for EFS and OS, using a limited backward elimination procedure.

Pathway analysis

KEGG pathways were analyzed by cluster-profiler package [32]. Given a ranked list of genes, gene set enrichment analysis (GSEA) determines whether predefined set of *CCT3* is disproportionally overrepresented in the top or the bottom of the list instead of randomly across the list [33, 34].

Different expression genes analysis

Limma package was used to identify different expression genes (DEGs) [32]. The statistical cutoff values were a fold-change of 1.0 and an adjusted *P* value of ≤ 0.05. All analyses were performed using R 3.5.0.
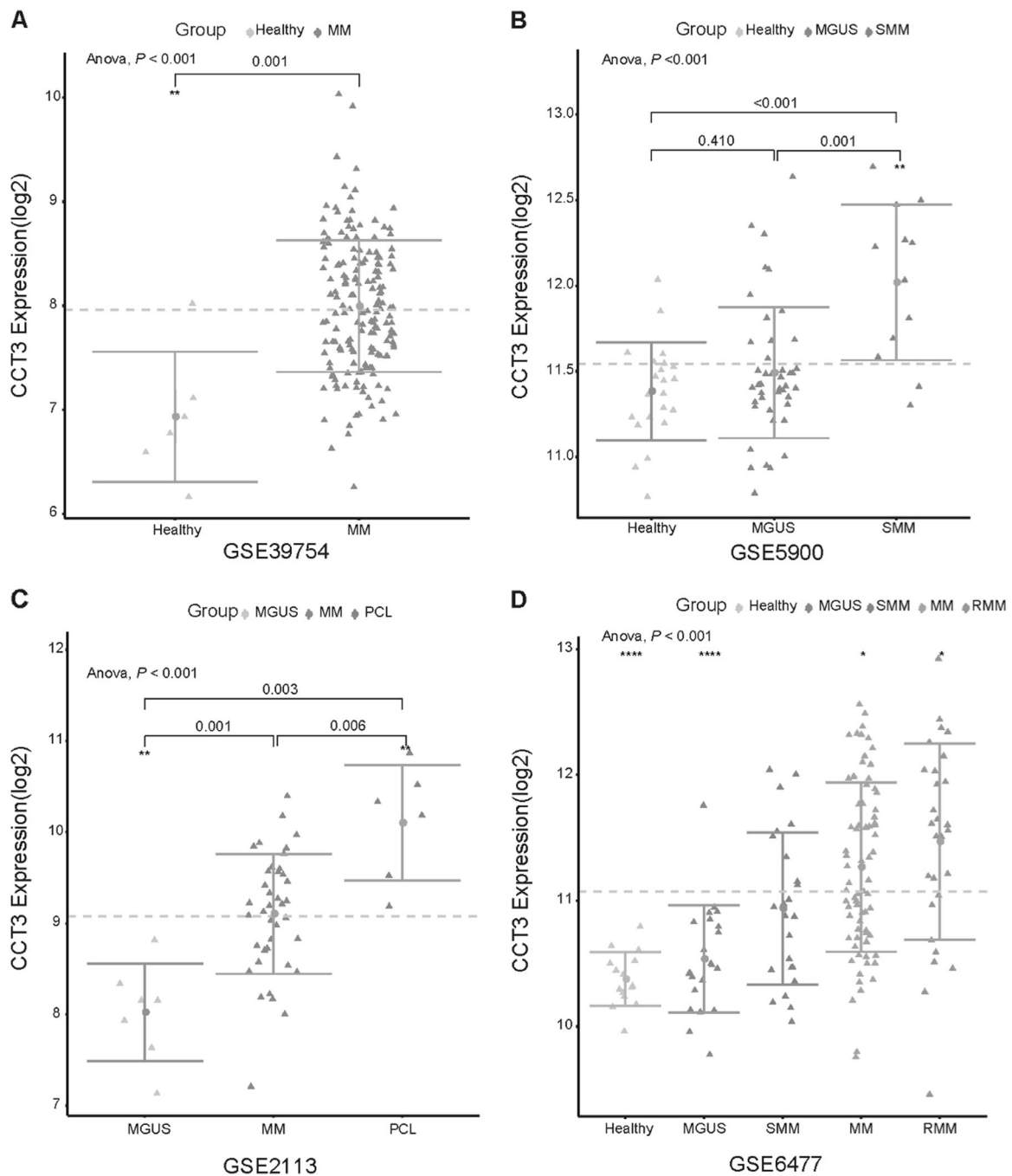
Fig. 1 MM progression was associated with the expression of CCT3. a MM patients ($n = 170$) have higher *CCT3* expression than the healthy donors ($n = 6$). b SMM patients ($n = 12$) have higher *CCT3* expression than MGUS ($n = 44$) patients. c *CCT3* expression increased gradually from MGUS ($n = 7$), MM ($n = 39$) to PCL ($n = 6$). d *CCT3* expression increased gradually from MGUS ($n = 22$), SMM ($n = 24$), MM ($n = 69$) to relapse MM (RMM, $n = 28$).

Results

The increased expression of *CCT3* was associated with MM progression

We firstly analyzed the different expression of *CCT3* in four independent datasets (GSE39754, GSE5900, GSE2113, and GSE6477). We found the expression of *CCT3* in MM patients ($n = 170$) was significantly higher than the healthy donors ($n = 6$) (GSE39754, $P = 0.0012$) (Fig. 1a). Overexpression of *CCT3* was further validated by GSE5900 dataset samples including 22 healthy donors, 44 MGUS, and 12 SMM patients, with $P < 0.001$, $P = 0.410$, $P = 0.001$, respectively (Fig. 1b). Furthermore, a third database,

including 7 MGUS, 39 MM and 6 PCL, was used for the *CCT3* expression analysis (GSE2113). And it showed a significant increased expression of *CCT3* in MGUS, MM and PCL gradually ($P = 0.003$, 0.001, 0.006, respectively) (Fig. 1c). Moreover, the expressions of *CCT3* in healthy donor ($n = 15$), MGUS ($n = 22$), SMM ($n = 24$), MM ($n = 69$) and RMM ($n = 28$) increased gradually (GSE6477) (Fig. 1d). These results indicated that *CCT3* expression level was coincidence with the myeloma progression.

We further investigate whether the expression of *CCT3* was related to different types of MM. The expression of CCT3 decreased in turn in patients with t(14;16) ($n = 4$), t (4;14) ($n = 7$), RB deletion ($n = 14$), normal ($n = 13$) and t (11;14) ($n = 11$) karyotypes, and most of the patients have higher *CCT3* expression than healthy donor except for patients with t(11;14) karyotypes (Fig. 2a). In MM patients, the expression of *CCT3* was higher at relapse than at initial diagnosis (GSE82307, $n = 166$, $P = 0.014$) (Fig. 2b). In our study, we found that *CCT3* expressed higher in patients with B2M ≥ 3.5 mg/L and LDH ≥ 250 U/L compared with those with B2M < 3.5 mg/L and LDH < 250 U/L (GSE38627 dataset, $n = 130$) (Fig. 2c, d).

High *CCT3* expression was associated with adverse outcomes in MM patients

In the GSE24080 ($n = 559$) microarray dataset, *CCT3*^{high} patients had a significantly shorter EFS and OS (all $P < 0.001$) than *CCT3*^{low} patients (Fig. 3a, b). In the GSE2658 ($n = 559$) and GSE4204 ($n = 538$) datasets, the *CCT3*^{high} group had markedly shorter OS compared with the *CCT3*^{low} group ($P < 0.001$, $P < 0.001$ respectively) (Fig. 3c). In the GSE4204 dataset, *CCT3*^{high} patients had a significantly shorter OS ($P < 0.001$) than *CCT3*^{low} patients (Fig. 3d). All these data suggested high expression of *CCT3* was a dismal prognosis factor.

CCT3 expression related clinical and molecular characteristics

The clinical and molecular characteristics of *CCT3*^{high} and *CCT3*^{low} were compared respectively (Table 1). *CCT3*^{high} group had more abnormal cytogenetic, more high expression of *CDK4*, *IDH2*, and *TP53* and less high expression of *CCND1* and *FGFR3*. In addition, *CCT3*^{high} group had higher B2M, CRP, LDH, ASPC, and BMCP, and lower HGB. No significant differences were found in age, gender, race, therapy, CREAT, ALB, MRI, isotype and high expression of *LIG4* (Table 1).

Multivariate analysis was used to further assess the prognostic value of *CCT3* (Table 2). The result indicated that high *CCT3* expression, high B2M and high LDH were independent unfavorable prognostic factors for OS ($P =$

0.001, 0.039, and $P < 0.001$, respectively). High *CCT3* expression, high LDH, and HGB were independent risk factors for EFS ($P = 0.028$, 0.004, and 0.038, respectively).

CCT3 expression related signaling pathways in MM

We utilized the KEGG and GSEA enrichment analysis to investigate the possible regulation mechanisms of *CCT3* expression in the process of MM. KEGG pathway analysis indicated that the *CCT3* targeted genes were involved in JAK-STAT3 signaling pathway, Hippo signaling pathway, WNT signaling pathway and two pathways centralizing in leukemia related terms (namely acute myeloid leukemia and chromic myeloid leukemia) (Fig. 4a). Consistent with the KEGG finding, GSEA of gene sets differentially regulated in *CCT3*^{high} and *CCT3*^{low} groups revealed that the leukocyte migration, regulation of leukocyte migration, IL6/JAK/ STAT3 signaling and regulation of STAT cascade gene sets were significantly upregulated in *CCT3*^{high} group. These finding indicated that high expression of *CCT3* was related to the leukemia and JAK-STAT3 signaling pathway.

Associations between genome-wide expression profiles and *CCT3* expression

By analyzing the GSE24080 dataset ($n = 559$), 18 different expression genes (DEGs) were identified between *CCT3*^{high} ($n = 280$) and *CCT3*^{low} ($n = 279$), including 8 upregulated genes and 10 downregulated genes that were significantly associated with *CCT3* expression (|logFC| > 1, P value < 0.05) (Fig. 5a). The upregulated genes included *CCT3*, *MYC*, *NVL*, *FABP5*, *MATR3*, *NUF2*, *LAMP5*, and *MEIS2*. The downregulated genes included *CCND1*, *BASP1*, *PF4*, *PPBP*, *MDK*, *SLC2A10*, *CD163*, *VCAM1*, *SLC46A3*, and *CTSW*.

For further, the different expressed genes were in a protein–protein interaction (PPI) network analysis. *CCT3* can directly function with *FABP5*, *CCND1 and MATR3*, and *CCT3* can regulate *MYC* through *FABP5* (Fig. 5b). The different expression values were validated in the heatmaps of the 18 DEGs in GSE24080 (Fig. 5c).

Discussion

In our present study, by performing different gene expression analysis, survival analysis, and other bioinformatics analytical methods using ten independent GEO datasets, we identified high *CCT3* was an adverse progression of MM. And *CCT3* was significantly overexpressed in MM patients compared with healthy donors in our study (Fig. 1), indicating that it may be a carcinogenic protein.
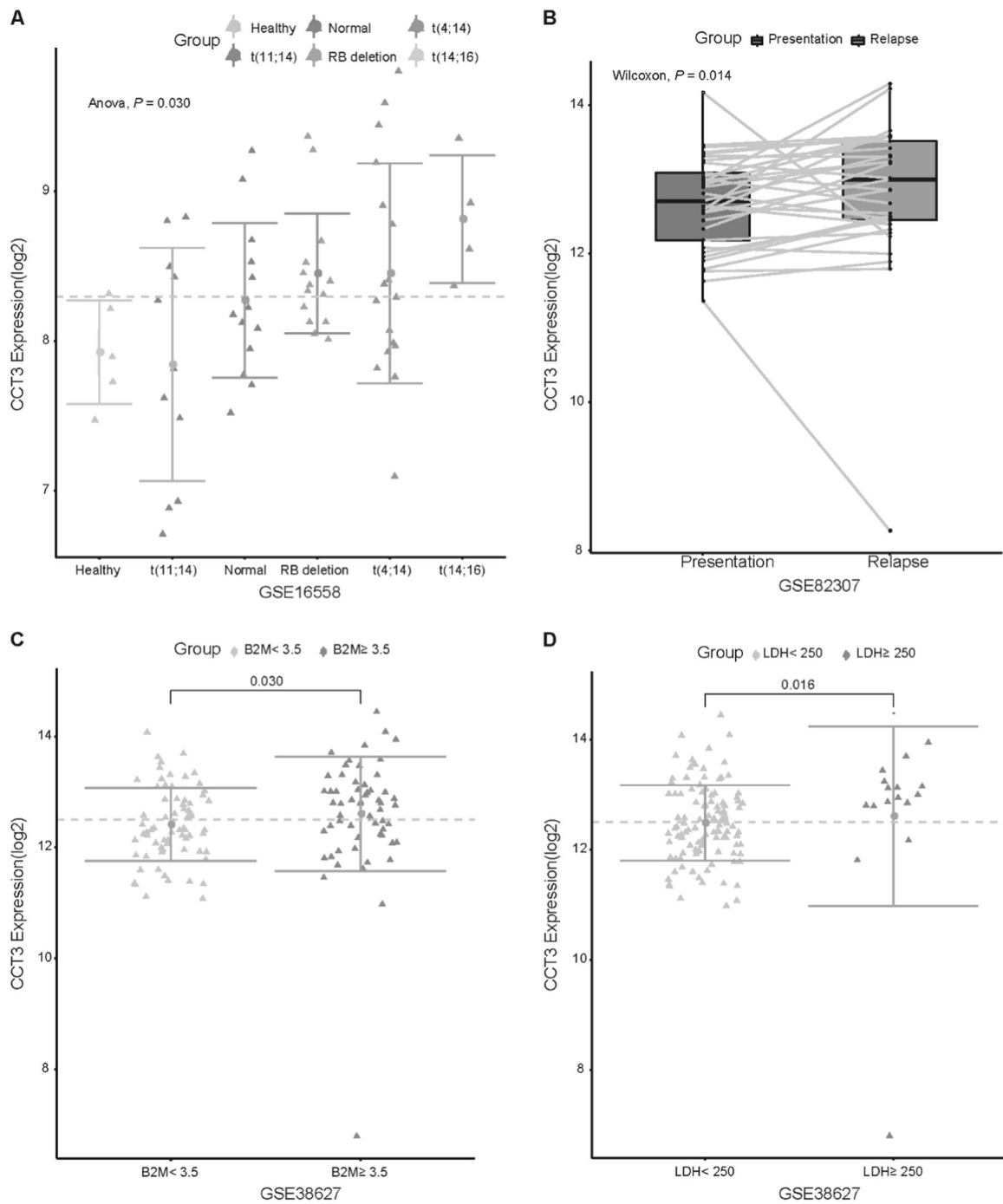
Fig. 2 The expression level of *CCT3* was related to different types of MM. a The expression of *CCT3* decreased in turn in patients with t (14;16) ($n = 4$), t(4;14) ($n = 7$), RB deletion ($n = 14$), normal ($n = 13$) and t(11;14) ($n = 11$) karyotypes. **b** The expression of *CCT3* was higher at relapse than at initial diagnosis ($n = 33$). **c** The expression of *CCT3* was higher in patients with B2M ≥ 3.5 mg/L ($n = 59$) compared with those with B2M < 3.5 mg/L ($n = 71$). **d** The expression of *CCT3* was higher in patients with LDH ≥ 250 U/L ($n = 16$) compared with those with LDH < 250 U/L ($n = 114$).

Previous research has proved that the abnormal expression of *CCT3* can affect the migration of cancer cells and the prognosis of cancer patients. Altered expression of CCT3 was found in many cancers, such as hepatocellular carcinoma (HCC), gastric cancer, liver cancer and colorectal cancer [8, 9, 35, 36], and overexpression of *CCT3* was not only associated with lymph-node metastasis of gastric cancer [35] but also can indicate a poor prognosis in HCC patients [37, 38]. And in line with the above findings, high expression of *CCT3* was an adverse prognosis factor in our
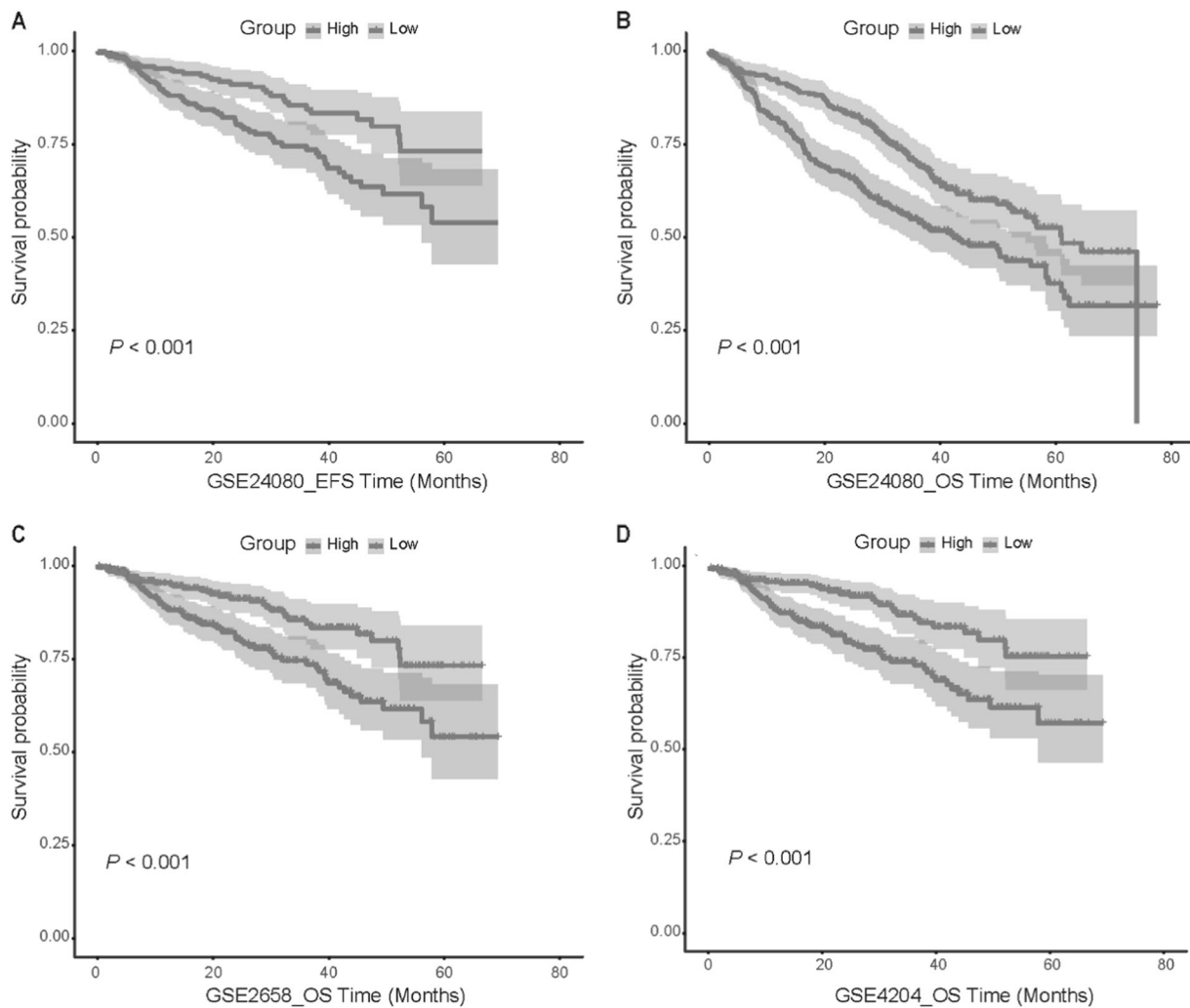
Fig. 3 High *CCT3* expression was associated with adverse outcomes in MM patients. a In GSE24080 ($n = 559$) dataset, *CCT3*^{high} patients had a significantly shorter EFS ($P < 0.001$) than *CCT3*^{low} patients. **b** In GSE24080 dataset, *CCT3*^{high} patients had a significantly shorter OS ($P < 0.001$) than *CCT3*^{low} patients. **c** In GSE2658 ($n = 559$) dataset, CCT3^{high} patients had a significantly shorter OS ($P < 0.001$) than *CCT3*^{low} patients. **d** In GSE4204 ($n = 538$) dataset, *CCT3*^{high} patients had a significantly shorter OS ($P < 0.001$) than *CCT3*^{low} patients.

study. The expression level of CCT3 increased gradually with the progress of disease from MGUS, SMM, MM to RMM and PCL (Fig. 1b–d). With the malignancy of the disease, the expression of *CCT3* also increased gradually (Fig. 1c). Different karyotypes of MM also predict different prognosis. In our study, MM with adverse abnormality translocations (including t(14;16), t(4;14) and RB deletion) have higher *CCT3* expression, and MM with favorable abnormality translocations of t(11;14) have relatively lower CCT3 expression (Fig. 2a). And *CCT3* was highly expressed in relapse than in detection of MM patients (Fig. 2b). In addition, serum beta-2 microglobulin (B2M) < 3.5 mg/L and serum LDH ≤ the upper limit of normal are used to define the stage I of MM (Guideline). Furthermore, high *CCT3* expression was proved to be a bad survival predictor by

analyzing three independent datasets (Fig. 2c, d). These finding indicated that high expression of *CCT3* may serve as an indicator in diagnosis and prognosis of MM patients.

Clinical and characters of high and low *CCT3* expression groups were analyzed in our study. *CCT3*^{high} group had higher B2M and LDH. These characters were proved to be strong predictors for MM patients [39, 40]. And in multivariate analysis, *CCT3*, B2M and LDH were proved to be independent negative prognosis factors for OS in MM patients. This result confirmed the inferior effect of *CCT3* on MM for further.

CCT3 affects the progression of HCC by activating signal transducer and activator of transcription (STAT)3/ STAT3 [37, 41]. STAT3 is the major factor in JAK-STAT3 pathway signaling, which plays an important role in many

Table 1 Patients' characteristics of 559 MM patients according to *CCT3* expression levels in GSE24080.

	CCT3[low], $n = 279$	*CCT3*^{high}, $n = 280$	*P* value
AGE, mean (range)	57.41 (24.83–76.50)	58.57 (29.70–75.94)	0.160[a]
Gender (%)			
Female	101 (36.2)	121 (43.21)	0.108[b]
Male	178 (63.8)	159 (56.79)	
Race (%)			
Other	31 (11.11)	31 (11.07)	1.000[b]
White	248 (88.89)	249 (88.93)	
Cytogenetic abnormality (%)			
No	209 (74.91)	143 (51.07)	<0.001[b]
Yes	70 (25.09)	137 (48.93)	
Therapy (%)			
TT2	166 (59.5)	179 (63.93)	0.323[b]
TT3	113 (40.5)	101 (36.07)	
B2M (mean (SD))	4.098 (4.835)	5.363 (5.785)	0.005[c]
CRP (mean (SD))	9.364 (15.627)	13.888 (28.221)	0.019[c]
CREAT (mean (SD))	1.24 (1.131)	1.405 (1.391)	0.125[c]
LDH (mean (SD))	157.921 (52.804)	185.982 (74.308)	<0.001[c]
ALB (mean (SD))	4.082 (0.554)	4.015 (0.608)	0.173[c]
HGB (mean (SD))	11.574 (1.725)	10.934 (1.842)	<0.001[c]
ASPC (mean (SD))	39.426 (23.353)	45.913 (23.683)	0.001[c]
BMPC (mean (SD))	43.243 (25.552)	49.475 (25.919)	0.004[c]
MRI (mean (SD))	10.431 (14.485)	11.637 (13.591)	0.310[c]
ISOTYPE (%)			
FLC	45 (16.13)	39 (13.93)	0.632[b]
IgA	59 (21.15)	74 (26.43)	
IgG	160 (57.35)	153 (54.64)	
High *CCND1*, no (%)	166 (59.5)	114 (40.71)	0.008[b]
High *CDK4*, no (%)	102 (36.56)	178 (63.57)	0.001[b]
High *LIG4*, no (%)	150 (53.76)	130 (46.43)	0.730[b]
High *FGFR3*, no (%)	157 (56.27)	123 (43.93)	0.002[b]
High *IDH2*, no (%)	125 (44.8)	155 (55.36)	0.028[b]
High *TP53*, no (%)	115 (41.22)	165 (58.93)	0.001[b]

AGE age at registration (years), *Cytogenetic abnormality* an indicator of the detection of cytogenetic abnormalities, *B2M* beta-2 microglobulin, mg/l, *CRP* C-reactive protein, mg/l, *CREAT* creatinine, mg/dl, *LDH* lactate dehydrogenase, U/l, *ALB* albumin, 35 g/l, *HGB* hemoglobin, g/dl, *ASPC* aspirate plasma cells (%), *BMPC* bone marrow biopsy plasma cells (%), *MRI* number of magnetic resonance imaging (MRI)-defined focal lesions (skull, spine, pelvis), *SD* standard deviation, *no* number of patients

[a]Cruskal–Wallis test

[b]Chi-square test

[c]Welch Two Sample *t*-test

aspects of tumorigenesis [42]. By binding specific enhancers, STAT dimers can regulate the transcription of target genes [43]. The activation of STAT dimers in nucleus can be affected by mitogen-activated protein kinase (MAPK), AKT/mammalian target of rapamycin (mTOR) and JAK [44], and a recent study disclosed that mTORC, which is multi-protein signaling complex of mTOR, assembly and signaling can be affect by eukaryotic chaperonin CCT [45].

Here we studied the possible mechanism pathways of *CCT3* in MM. In our study, high *CCT3* expression was associated with JAK-STAT3 rather than mTOR pathway by KEGG and GSEA analysis (Fig. 4). And through different expression gene analysis and PPI analysis, we found that MYC was highly expressed in *CCT3*^{high} group. c-MYC, as a target gene in cancer, has been proved to be implicated in STATs [46]. And in our study, MYC was upregulated in

Table 2 Multivariate analysis for EFS and OS.

Variables	EFS		OS	
	HR (95%CI)	P value	HR (95%CI)	P value
CCT3 (High vs. Low)	1.49 (1.04–2.13)	0.028	1.87 (1.31–2.67)	0.001
B2M	1.15 (0.79–1.69)	0.461	1.50 (1.02–2.21)	0.039
CRP	0.83 (0.59–1.17)	0.282	1.24 (0.90–1.72)	0.192
LDH	2.11 (1.27–3.50)	0.004	2.46 (1.62–3.73)	<0.001
HGB	0.68 (0.47–0.98)	0.038	0.95 (0.66–1.35)	0.760
ASPC	0.97 (0.66–1.44)	0.887	1.32 (0.89–1.96)	0.173
BMPC	1.53 (1.00–2.34)	0.052	1.28 (0.83–1.98)	0.267
CCND1 (High vs. Low)	0.77 (0.56–1.08)	0.126	0.93 (0.67–1.28)	0.645
CDK4 (High vs.Low)	1.01 (0.73–1.41)	0.951	1.19 (0.85–1.65)	0.312
FGFR3 (High vs. Low)	1.07 (0.78–1.48)	0.663	0.99 (0.72–1.35)	0.925
MYC (High vs. Low)	0.83 (0.59–1.18)	0.305	0.84 (0.59–1.17)	0.299
TP53 (High vs. Low)	1.03 (0.74–1.44)	0.869	0.78 (0.56–1.08)	0.139

EFS event-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval, *B2M* Beta-2microglobulin, mg/l, *CRP* C-reactive protein, mg/l, *LDH* lactate dehydrogenase, U/l, *HGB* hemoglobin, g/dl, *ASPC* aspirate plasma cells (%), *BMPC* bone marrow biopsy plasma cells (%)
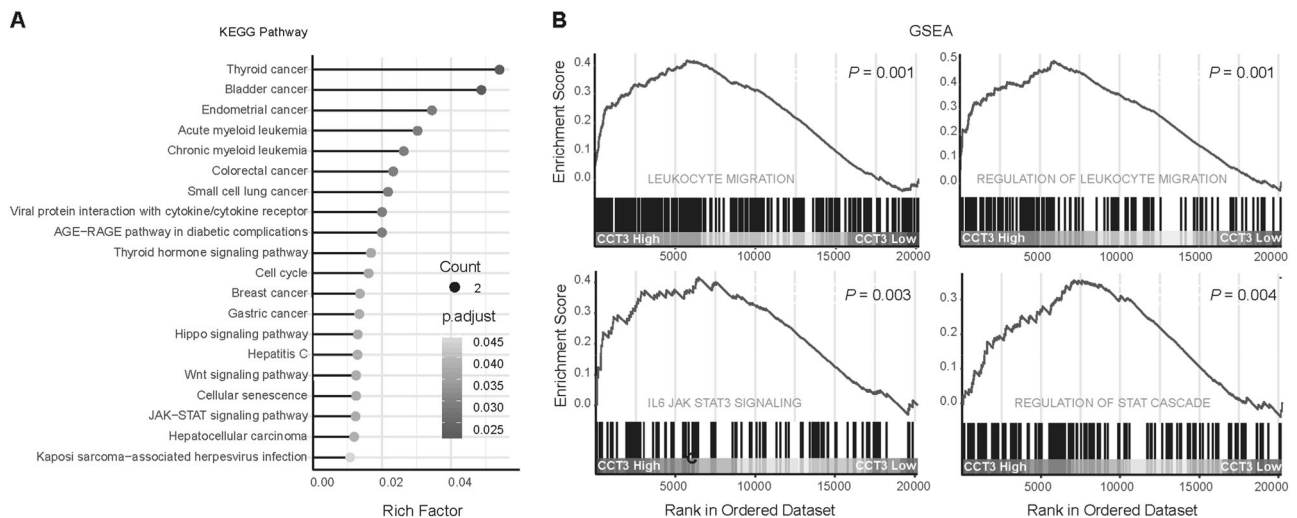

Fig. 4 *CCT3* **expression related signaling pathways in MM. a** KEGG analysis revealed that *CCT3* targeted genes were involved in JAK-STAT3 signaling pathway, Thyroid hormone signaling pathway, Hippo signaling pathway, WNT signaling pathway and two pathways centralizing in leukemia related terms (namely acute myeloid leukemia and chromic myeloid leukemia). **b** GSEA analysis revealed that *CCT3* high expression was associated with upregulated pathways of leukocyte migration, regulation of leukocyte migration, IL6/JAK/STAT3 signaling and regulation of STAT cascade.

MM patients with high *CCT3* expression (Fig. 5a) and was connected with *CCT3* through *FABP5* (Fig. 5b). All these findings indicated that high *CCT3* can affect the progression of MM through JAK-STAT3 signaling pathway. In addition, *CCT3* expression was also related to the Hippo signaling pathway and WNT signaling pathway (Fig. 4a), which are closely related to carcinogenesis [47–49]. Therefore, *CCT3* expression may influence the progress of MM through these three signaling pathways, and mainly by JAK-STAT3 signaling pathway.

In conclusion, increased expression level of *CCT3* was more likely to express in dangerous MM patients and can independently predict an adverse prognosis for MM patients. High expression of *CCT3* may be a potential candidate biomarker for the molecular diagnosis and prognosis of MM patients. Otherwise, *CCT3* expression was associated with Hippo signaling pathway, WNT signaling pathway, and JAK-STAT3 signaling pathway. Overexpression of *CCT3* may influence the MM progress mainly through JAK-STAT3 signaling pathway, and *CCT3* can be used as a potential therapy target in MM in the future.
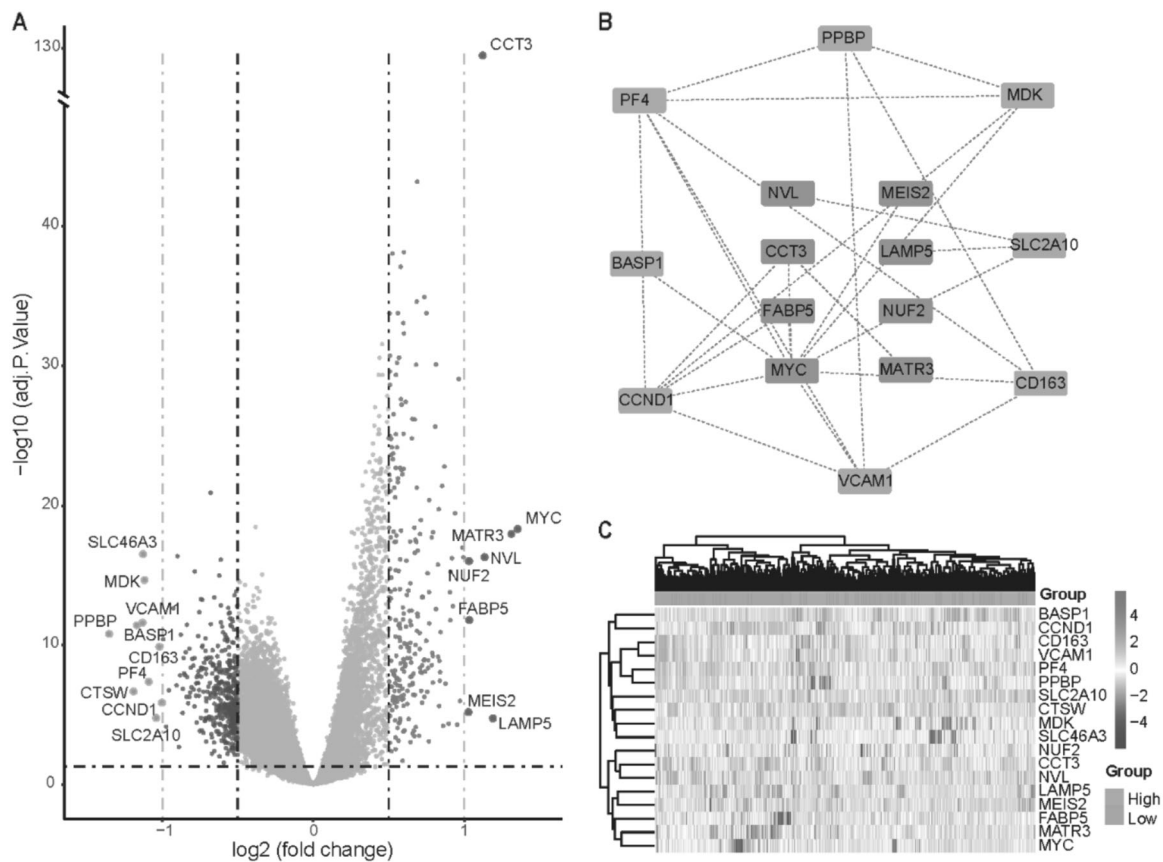
Fig. 5 Different expression genes (DEGs) between *CCT3*^high and *CCT3*^low group in MM patients. a Volcano plots of DEGs between *CCT3*^high and *CCT3*^low group. From GSE24080 dataset ($n = 559$), 18 DEGs were identified between *CCT3*^high ($n = 280$) and *CCT3*^low ($n = 279$), including 8 upregulated genes (purple dots) and 10 downregulated genes (green dots) that were significantly associated with CCT3 expression ($|logFC| > 1$, $P < 0.05$). **b** PPI network of the DEGs. The red points represent upregulated genes and green points represent downregulated genes. **c** Heatmaps showed that 18 DEGs between *CCT3*^high and *CCT3*^low group of GSE24080.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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