

## RESEARCH ARTICLE

# Long non-coding RNA T-cell factor 7 in multiple myeloma: A potential biomarker for deteriorated clinical features and poor prognosis

Cui Zhang | Min Chu | Yingchao Fan | Liting Wu | Zhumeng Li | Xiaoyan Ma |  
Wenfang Zhuang 

Department of Medical Laboratory, Shidong Hospital of Yangpu District, Shanghai, China

**Correspondence**

Wenfang Zhuang, Department of Medical Laboratory, Shidong Hospital of Yangpu District, 999 Shiguang Road, Shanghai, 200438, China.  
Email: wozhuang8210112@163.com

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**Abstract**

**Background:** This study aimed to investigate the correlation of long non-coding RNA T-cell factor 7 (lnc-TCF7) with clinical features and prognosis in patients with multiple myeloma (MM).

**Methods:** Totally, 216 newly diagnosed symptomatic MM patients and 60 healthy controls (HCs) were enrolled. Bone marrow samples were collected from patients before treatment and from HCs on donation to detect lnc-TCF7 expression in plasma cells by reverse transcription quantitative polymerase chain reaction. Besides, clinical response, progression-free survival (PFS), and overall survival (OS) of patients were assessed.

**Results:** lnc-TCF7 expression was increased in patients with MM compared with HCs. lnc-TCF7 expression was highest in international staging system (ISS) stage III patients, followed by ISS stage II patients, and then ISS stage I patients, while lnc-TCF7 expression was similar in patients with different immunoglobulin subtypes and Durie-Salmon stages. Regarding chromosomal abnormalities, lnc-TCF7 expression positively correlated with t(4; 14) and Del(17p), whereas no correlation of lnc-TCF7 expression with t(14; 16), 1q21 amplification, Del(13q), or hyperdiploid was observed in patients with MM. Furthermore, lnc-TCF7 expression positively correlated with serum creatinine, beta-2-microglobulin, and lactate dehydrogenase in patients. Besides, lnc-TCF7 was negatively associated with complete response but not overall response rate in patients. Additionally, patients with lnc-TCF7 high expression exhibited shorter PFS and OS compared to patients with lnc-TCF7 low expression.

**Conclusion:** lnc-TCF7 might have clinical value in aiding disease management and prognosis prediction of MM.

**KEYWORDS**

clinical response, long non-coding RNA TCF7, multiple myeloma, overall survival, progressive-free survival

Cui Zhang and Min Chu contributed equally to this work.

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## 1 | INTRODUCTION

Multiple myeloma (MM), the second most common hematological malignancy, is characterized by abnormal proliferation of monoclonal plasma cells in bone marrow and overproduction of monoclonal immunoglobulin in serum and/or urine with an estimated 159 985 new cases and 106 105 deaths worldwide in 2018.<sup>1,2</sup> The typical symptoms of MM are bone pain, hypercalcemia, anemia, and renal insufficiency.<sup>3</sup> Over the last two decades, the introduction of immunomodulatory agents, proteasome inhibitors, monoclonal antibodies, and histone deacetylase inhibitor has improved the depth and duration of treatment responses and prolonged survival in patients with MM.<sup>4,5</sup> Unfortunately, a considerable number of patients eventually suffer from disease recurrence and/or respond poorly to these treatments.<sup>6</sup> Therefore, the exploration of potential biomarkers is necessary for disease risk assessment, disease monitoring, and prognosis prediction of MM.

Long non-coding RNAs (lncRNAs), a class of non-coding RNAs, consist of more than 200 nucleotides with no open reading frame.<sup>7</sup> Certain lncRNAs such as lncRNA UCA 1 and lncRNA XLOC\_013703 have been proposed as oncogenes or tumor suppressors in multiple cancers.<sup>8-10</sup> Long non-coding RNA T-cell factor 7 (lnc-TCF7), a recently identified lncRNA, is involved in the tumorigenesis of solid tumors such as colorectal cancer and liver cancer.<sup>11-13</sup> For example, knockdown of lnc-TCF7 suppresses the migration and invasion of colorectal cancer cells.<sup>12</sup> Another study illuminates that lnc-TCF7 facilitates the self-renewal of liver cancer stem cells.<sup>13</sup> In clinical studies, lnc-TCF7 is associated with poor prognosis in patients with colorectal cancer and glioma.<sup>14,15</sup> Additionally, it is shown that lnc-TCF7 activates Wnt signaling pathways through the recruitment of the Switch/Sucrose non-fermentable complex to T-cell factor 7 (TCF7) promoter.<sup>13</sup> Meanwhile, Wnt signaling pathway functions as an important regulator for cell differentiation, proliferation, and apoptosis of MM.<sup>16</sup> Based on these perceptions, we speculated that lnc-TCF7 might have clinical implications in the progression and prognosis of MM. However, no related studies have been reported yet. Therefore, we investigated the correlation of lnc-TCF7 with clinical features and prognosis in patients with MM, aiming to provide a new perspective for lnc-TCF7 in assisting personalized treatments and improving prognosis of MM.

## 2 | METHODS

### 2.1 | Participants

A total of 216 newly diagnosed symptomatic MM patients were consecutively enrolled in Shidong Hospital of Yangpu District from July 2015 to June 2019. The inclusion criteria in this study were as follow: (a) diagnosed as de novo symptomatic MM according to International Myeloma Working Group (IMWG) criteria of multiple myeloma<sup>17</sup>;

(b) age above 18 years old; and (c) without other hematologic malignancies or solid tumors. The exclusion criteria were (a) smoldering (asymptomatic) myeloma; (b) plasmablastic lymphoma; (c) history of chemotherapy or radiotherapy; (d) history of stem cell transplantation; and (e) pregnant or lactating women. Besides, 60 healthy bone marrow donors were recruited as healthy controls (HCs) between July 2015 and August 2019. This study was approved by the Institutional Review Board of Shidong Hospital of Yangpu District. All participants or their guardians provided the written informed consents before enrollment.

### 2.2 | Data collection

The clinical characteristics of patients were collected after the written informed consents were obtained, which included: (a) demographic characteristics (age and gender); (b) immunoglobulin subtype (eg, immunoglobulin G (IgG), and immunoglobulin A (IgA) and other subtypes); (c) bone status (eg, bone lesion); (d) renal function status (eg, renal impairment); (e) biochemical indexes (eg, hemoglobin (Hb), calcium, serum creatinine (Scr), albumin (ALB), beta-2 microglobulin ( $\beta$ 2-MG), and lactate dehydrogenase (LDH)); (f) chromosomal abnormalities (eg, t(4; 14), t(14; 16), and Del (17p)); and (g) clinical stage (Durie-Salmon stage and ISS stage).<sup>18,19</sup>

### 2.3 | Sample collection

Bone marrow samples of patients were collected before treatment, and bone marrow samples of HCs were obtained on donation. After collection, the bone marrow samples were separated for bone marrow mononuclear cells using gradient density centrifugation. Then, CD138-positive plasma cells were isolated from mononuclear cells using CD138-coated magnetic beads (Miltenyi Biotec), and the procedure was consistent with the manufacturer's instructions. The relative expression of lnc-TCF7 in plasma cells was detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

### 2.4 | RT-qPCR

Firstly, total RNA was isolated from CD138-positive plasma cells using PureZOL RNA isolation reagent (Bio-Rad). Then, RNA was reverse-transcribed into cDNA using iScript™ cDNA Synthesis Kit (Bio-Rad). Subsequently, lncRNA TCF7 relative expression was determined by performing polymerase chain reaction with KOD SYBR® qPCR Mix (Toyobo). lncRNA TCF7 relative expression was calculated by  $2^{-\Delta\Delta Ct}$  method using GAPDH as internal reference. Sequences of primers used were as follow: lnc-TCF7, forward: 5' GAAGCCCGTATTAGACTGAATGGT 3', reverse: 5' TTG AGACAATCTGGTATGAGGACAC 3'; GAPDH, forward: 5' GACCACA GTCCATGCCATCAC 3', reverse: 5' ACGCCTGCTTCACCACCTT 3'.

## 2.5 | Treatment

After enrollment, all patients received appropriate treatment regimens according to their clinical status and guideline for MM,<sup>20</sup> which included: (a) bortezomib/dexamethasone (BD) (bortezomib at a dose of 1.3 mg/m<sup>2</sup> on day 1, day 4, day 8, day 11; dexamethasone at a dose of 20 mg/d on day 1, day 2, day 4, day 5, day 8, day 9, day 11, day 12; 21 days as a cycle); (b) bortezomib/cyclophosphamide/dexamethasone (BCD) (bortezomib at a dose of 1.3 mg/m<sup>2</sup> on day 1, day 4, day 8, day 11; cyclophosphamide at a dose of 140 mg/m<sup>2</sup> and dexamethasone at a dose of 20 mg/d on day 1-4; 21 days as a cycle); (c) lenalidomide/low-dose dexamethasone (Rd) (lenalidomide at a dose of 25 mg/d for 21 days; dexamethasone at a dose of 20 mg/w; 28 days as a cycle); (d) melphalan/prednisone/bortezomib (MPB) (melphalan at a dose of 9 mg/m<sup>2</sup> and prednisone at a dose of 60 mg/m<sup>2</sup> on day 1-4; bortezomib at a dose of 1.3 mg/m<sup>2</sup> on day 1, day 4, day 8, day 11, day 22, day 25, day 29, and day 32; 42 days as a cycle); and (e) Bortezomib or Dexamethasone/cyclophosphamide/etoposide/cisplatin (DCEP) (dexamethasone at a dose of 30 mg/d, cyclophosphamide at a dose of 400 mg/m<sup>2</sup>, etoposide at a dose of 40 mg/m<sup>2</sup> and cisplatin at a dose of 10 mg/m<sup>2</sup> on day 1-4; 28 days as a cycle). After treatment for 2 to 4 cycles, clinical response of patients was assessed based on guideline for MM,<sup>20</sup> which included complete response (CR), very good partial response (VGPR), and partial response (PR). And overall response rate (ORR) was determined by combining the proportion of patients who achieved confirmed CR, VGPR, or PR during treatment. After clinical response assessment, subsequent therapies including autologous stem cell transplant, allogeneic stem cell transplant, or continue myeloma therapy were conducted for the patients according to guideline for MM.<sup>20</sup> DCEP, as a salvage treatment, was used for patients who did not respond to initial induction therapies (BD, BCD, Rd, or MPB).

## 2.6 | Follow-up

Intensive follow-up was carried out for all patients until June 30, 2019, and the median follow-up duration was 29.0 months ranging from 2.0 to 47.0 months. Progression-free survival (PFS) was measured from the date of initiation of treatment to the date of disease progression or death. Overall survival (OS) was measured from the date of initiation of treatment to the date of death. The patients not known whether the disease has progressed or whether they have died at the last follow-up were censored on the date last visit or on the date they were last known to be alive.

## 2.7 | Statistical analysis

Statistical analysis was performed using SPSS 22.0 (IBM), and figures were plotted using GraphPad Prism 7.00 (GraphPad Software). Continuous variables (age, Hb, calcium, Scr, ALB,  $\beta$ 2-MG, and LDH) were checked for normality by using the Kolmogorov-Smirnov test.

The normally distributed variables (age, Hb, and calcium) were presented as mean  $\pm$  standard deviation (SD); the non-normal distributed variables (Scr, ALB,  $\beta$ 2-MG and LDH) were presented as median (interquartile range, IQR). Categorical variable (gender, immunoglobulin subtype, bone status, renal function status, clinical stage, and chromosomal abnormalities) was displayed as count (percentage). Comparison between two groups was determined by Wilcoxon rank sum test, and comparison among groups was analyzed by Kruskal-Wallis H test. Correlation between two continuous variables was determined by Spearman's rank correlation test. PFS and OS were displayed using Kaplan-Meier curves, and the difference of PFS and OS between groups was determined by log-rank test. *P* value < .05 was considered as significant.

## 3 | RESULTS

### 3.1 | Characteristics of MM patients

The mean age of patients with MM was 57.8  $\pm$  8.8 years, and there were 83 (38.4%) females and 133 (61.6%) males. As for Durie-Salmon stage, 23 (10.6%) and 193 (89.4%) patients with MM had Durie-Salmon stages II and III, respectively. Regarding ISS stage, 44 (20.4%), 61 (28.2%), and 111 (51.4%) patients with MM were with ISS stage I, II, and III, respectively. In terms of chromosomal abnormalities, 22 (10.2%), 36 (16.7%), and 20 (9.3%) of patients with MM had t(4; 14), t(14; 16), and Del(17p) chromosomal abnormality, respectively. The detailed information of immunoglobulin subtype, bone status, renal function status, and biochemical indexes of patients with MM was listed in Table 1.

### 3.2 | Comparison of Inc-TCF7 between MM patients and HCs

The Inc-TCF7 relative expression was higher in patients with MM (Median: 2.732 [IQR: 1.708-4.342]) than that in HCs (Median: 1.053 [IQR: 0.412-1.710]) (*P* < .001) (Figure 1).

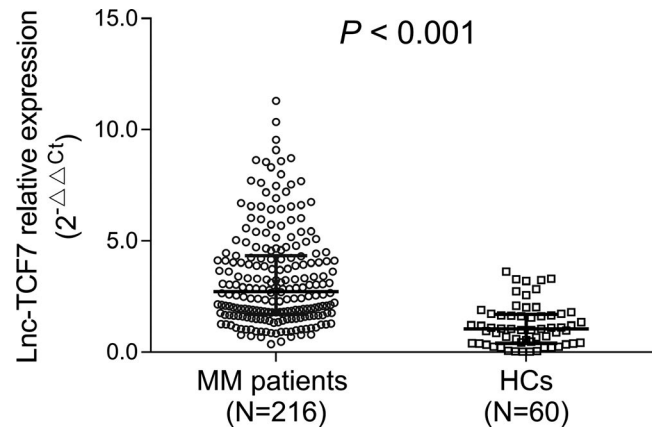
### 3.3 | Correlation of Inc-TCF7 with immunoglobulin subtype, Durie-Salmon stage, and ISS stage in MM patients

No difference of Inc-TCF7 relative expression was observed among MM patients with IgG, IgA, and other immunoglobulin subtypes (*P* = .127) (Figure 2A). And there was no difference of Inc-TCF7 relative expression between MM patients with Durie-Salmon stage II and III (*P* = .800) (Figure 2B). Whereas, as for ISS stage, Inc-TCF7 relative expression was the highest in MM patients with ISS stage III, followed by MM patients with ISS stage II, and then MM patients with ISS stage I (*P* < .001) (Figure 2C). These implied that Inc-TCF7 was correlated with advanced ISS stage in patients with MM.

**TABLE 1** Clinical characteristics of MM patients

Items	MM patients (N = 216)
Demographic characteristics	
Age (years), mean ± SD	57.8 ± 8.8
Gender, No. (%)	
Female	83 (38.4)
Male	133 (61.6)
Immunoglobulin subtype	
IgG, No. (%)	125 (57.9)
IgA, No. (%)	48 (22.2)
Others, No. (%)	43 (19.9)
Bone status	
Bone lesion, No. (%)	161 (74.5)
Renal function status	
Renal impairment, No. (%)	83 (38.4)
Clinical stage	
Durie-Salmon stage, No. (%)	
II	23 (10.6)
III	193 (89.4)
ISS stage, No. (%)	
I	44 (20.4)
II	61 (28.2)
III	111 (51.4)
Biochemical indexes	
Hb (g/L), mean ± SD	100.2 ± 24.8
Calcium (mg/dL), mean ± SD	9.9 ± 2.0
Scr (mg/dL), median (IQR)	1.9 (1.4-2.3)
ALB (g/L), median (IQR)	33.0 (29.0-37.0)
β2-MG (mg/L), median (IQR)	5.6 (2.9-9.5)
LDH (U/L), median (IQR)	208.2 (176.4-238.9)
Chromosomal abnormalities	
t(4; 14), No. (%)	22 (10.2)
t(14; 16), No. (%)	12 (5.7)
Del(17p), No. (%)	20 (9.3)
1q21 amplification, No. (%)	82 (38.0)
Del(13q), No. (%)	43 (29.6)
Hyperdiploid, No. (%)	96 (44.4)
Induction Treatments	
BD, No. (%)	82 (38.0)
BCD, No. (%)	35 (16.2)
Rd, No. (%)	59 (27.3)
MPB, No. (%)	40 (18.5)

Abbreviations: ALB, albumin; BCD, Bortezomib/cyclophosphamide/dexamethasone; BD, Bortezomib/dexamethasone; Hb, hemoglobin; IgA, immunoglobulin A; IgG, immunoglobulin G; IQR: interquartile range; ISS, International Staging System; LDH, lactate dehydrogenase; MM, multiple myeloma; MPB, Melphalan/prednisone/bortezomib; Rd, Lenalidomide/low-dose dexamethasone; Scr, serum creatinine; SD, standard deviation; β2-MG, Beta-2-microglobulin.



**FIGURE 1** The lnc-TCF7 relative expression in MM patients and HCs. The lnc-TCF7 relative expression between MM patients and HCs. Comparison of lnc-TCF7 relative expression between MM patients and HCs was assessed by Wilcoxon rank sum test.  $P < .05$  was considered significant. lnc-TCF7, long non-coding RNA T-cell factor 7; MM, multiple myeloma; HCs, healthy controls

### 3.4 | Correlation of lnc-TCF7 with chromosomal abnormality in MM patients

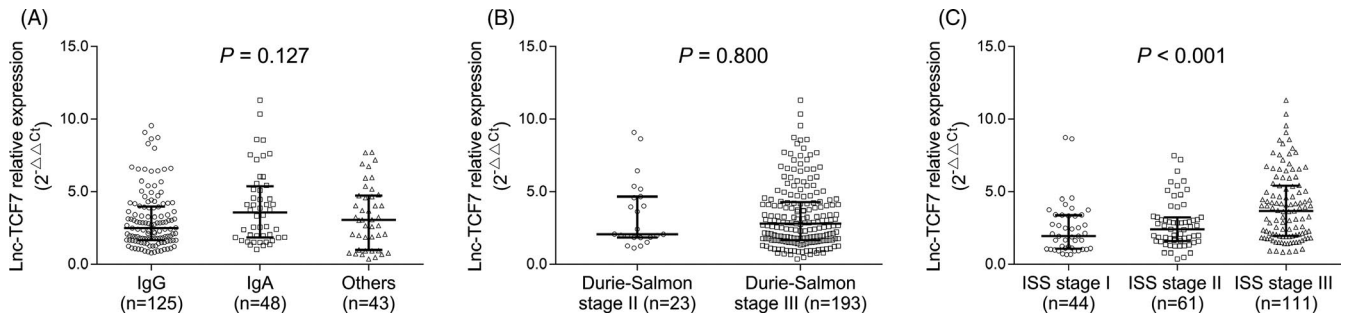
lnc-TCF7 relative expression was positively correlated with t(4; 14) ( $P = .006$ ) (Figure 3A) and Del(17p) ( $P = .034$ ) (Figure 3C), while no correlation of lnc-TCF7 relative expression with t(14; 16) ( $P = .116$ ) (Figure 3B), 1q21 amplification ( $P = .057$ ) (Figure 3D), Del(13q) ( $P = .271$ ) (Figure 3E), or hyperdiploid ( $P = .111$ ) (Figure 3F) was observed in patients with MM.

### 3.5 | Correlation of lnc-TCF7 with key biochemical indexes in MM patients

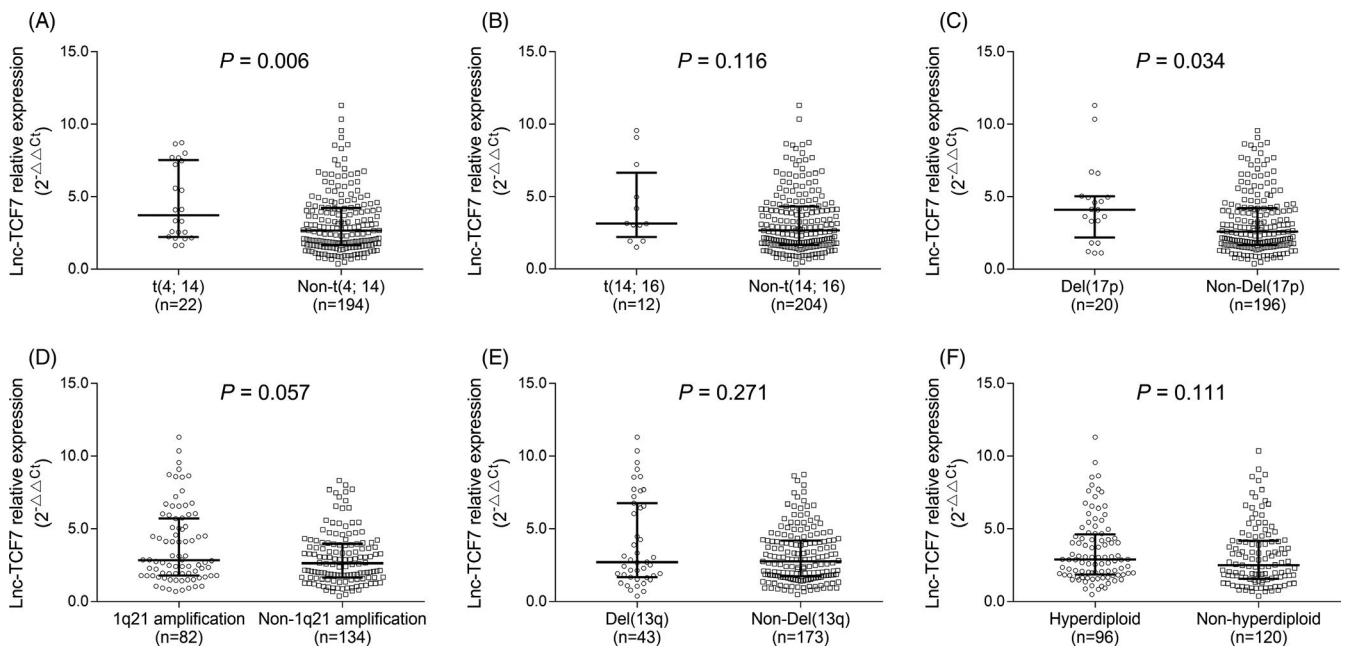
lnc-TCF7 relative expression was positively correlated with Scr ( $P = .012$ ,  $r = .170$ ), β2-MG ( $P < .001$ ,  $r = .336$ ), and LDH ( $P = .019$ ,  $r = .160$ ) in patients with MM (Table 2). Whereas, no correlation of lnc-TCF7 relative expression with Hb ( $P = .634$ ,  $r = .033$ ), calcium ( $P = .570$ ,  $r = -.039$ ), or ALB ( $P = .256$ ,  $r = -.078$ ) was observed in patients with MM (Table 2).

### 3.6 | Correlation of lnc-TCF7 with treatment response in MM patients

There were 56 (25.9%) and 162 (75.0%) patients with MM who achieved CR and ORR, respectively (Figure 4A). lnc-TCF7 relative expression was reduced in patients with MM who achieved CR compared to MM patients who did not achieve CR ( $P < .001$ ) (Figure 4B), while no difference of lnc-TCF7 relative expression was observed between MM patients who achieved ORR and MM patients who did not achieve ORR ( $P = .244$ ) (Figure 4C). These indicated that lnc-TCF7 was negatively correlated with CR in patients with MM.



**FIGURE 2** Comparison of lnc-TCF7 relative expression among MM patients with different immunoglobulin subtype, Durie-Salmon stage, and ISS stage. Comparison of lnc-TCF7 relative expression among MM patients with IgG, IgA, and other immunoglobulin subtypes was determined by Kruskal-Wallis H test (A). Comparison of lnc-TCF7 relative expression among MM patients with Durie-Salmon stage II and III was performed by Wilcoxon rank sum test (B). Correlation of lnc-TCF7 relative expression with ISS stage in MM patients was conducted by Spearman's rank correlation test (C).  $P < .05$  was considered significant. lnc-TCF7, long non-coding RNA T-cell factor 7; MM, multiple myeloma; ISS, international staging system; IgG, immunoglobulin G; IgA, immunoglobulin A; HCs, healthy controls



**FIGURE 3** Comparison of lnc-TCF7 among MM patients with different chromosomal abnormalities. Comparison of lnc-TCF7 relative expression between MM patients with t(4; 14) and MM patients with non-t(4; 14) (A). Comparison of lnc-TCF7 relative expression between MM patients with t(14; 16) and MM patients with non-t(14; 16) (B). Comparison of lnc-TCF7 relative expression between MM patients with Del(17p) and MM patients with non-Del(17p) (C). Comparison of lnc-TCF7 relative expression between MM patients with 1q21 amplification and MM patients with non-1q21 amplification (D). Comparison of lnc-TCF7 relative expression between MM patients with Del(13q) and MM patients with non-Del(13q) (E). Comparison of lnc-TCF7 relative expression between MM patients with hyperdiploid and MM patients with non-hyperdiploid (F). Comparison of lnc-TCF7 was assessed by Wilcoxon rank sum test.  $P < .05$  was considered significant. lnc-TCF7, long non-coding RNA T-cell factor 7; MM, multiple myeloma

### 3.7 | Percentage of patients received CR and percentage of patients received ORR under different treatment regimens

Under BD treatment, 24 (29.3%) and 65 (79.3%) patients with MM received CR and ORR, respectively (Figure S1). Under BCD treatment, 10 (28.6%) and 27 (77.1%) patients with MM received CR and ORR, respectively. Under Rd treatment, 11 (18.6%) and 39 (66.1%) patients with MM received CR and ORR, respectively. Under MPB

treatment, 11 (27.5%) and 29 (72.5%) patients with MM received CR and ORR, respectively.

### 3.8 | Correlation of lnc-TCF7 with survival profiles in MM patients

Patients with MM were divided into two groups, lnc-TCF7 high expression and lnc-TCF7 low expression, based on their median value of lnc-TCF7 relative expression (2.732). MM patients with lnc-TCF7



high expression exhibited shorter PFS than that in MM patients with Inc-TCF7 low expression ( $P < .001$ ) (Figure 5A). And MM patients with Inc-TCF7 high expression presented shorter OS than that in MM patients with Inc-TCF7 low expression ( $P < .001$ ) (Figure 5B). These implied that Inc-TCF7 was correlated with unfavorable survival profiles in patients with MM.

## 4 | DISCUSSION

In the present study, we discovered that (a) the Inc-TCF7 relative expression was elevated in MM patients compared to HCs; (b) Lnc-TCF7 positively correlated with ISS stage, t(4; 14), and Del(17p) chromosomal abnormality in patients with MM, and it was also positively associated with key biochemical indexes including Scr,  $\beta$ 2-MG, and LDH level; and (c) Lnc-TCF7 high expression was associated with lower CR and worse survival profiles in patients with MM.

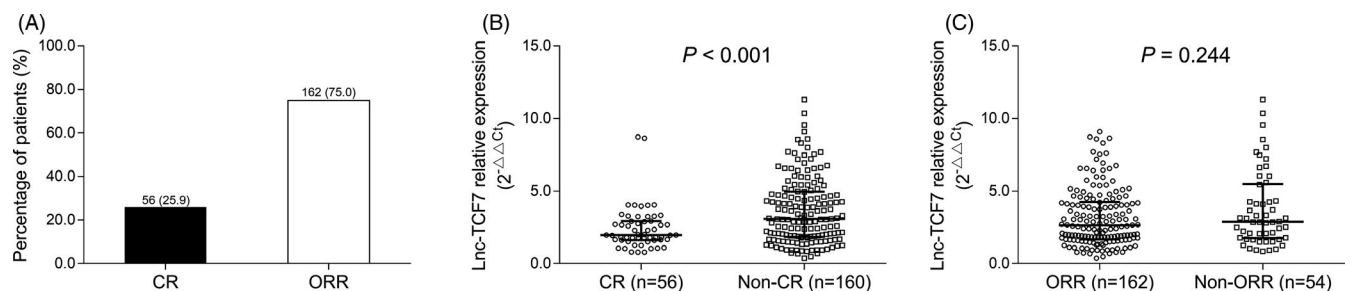
MM is a heterogenous disease with complex molecular biological characteristics.<sup>21</sup> Pathologically, malignant MM usually evolves from asymptomatic monoclonal gammopathy of underdetermined significance along with bone marrow microenvironment changes, genetic and cytogenetic abnormalities, which resulted

**TABLE 2** Correlation of Inc-TCF7 with biochemical indexes in MM patients

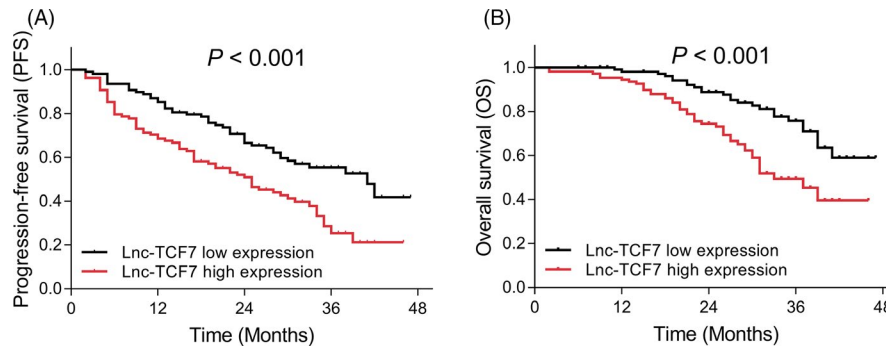
Items	Lnc-TCF7	
	P value	Correlation coefficient (r)
Hb	.634	.033
Calcium	.570	-.039
Scr	.012	.170
ALB	.256	-.078
$\beta$ 2-MG	<.001	.336
LDH	.019	.160

Note: Correlation was determined by Spearman's rank correlation test. Abbreviations: ALB, albumin; Hb, hemoglobin; LDH, lactate dehydrogenase; MM, multiple myeloma; Scr, serum creatinine;  $\beta$ 2-MG, Beta-2-microglobulin.

in end-organ damage, renal insufficiency, anemia, elevated bone marrow angiogenesis, and osteolytic bone lesions.<sup>3,22</sup> Due to the genetic clonal heterogeneity of MM, treatment of patients remains as one of the main challenges.<sup>6</sup> Therefore, it is urgent to explore the biomarkers for disease development and progression. Among the commonly investigated lncRNAs, Inc-TCF7 dysregulation is previously demonstrated to be associated with the onset and pathological features of several cancers.<sup>11,15</sup> For instance, Inc-TCF7 expression is upregulated in colorectal cancer tissues compared with adjacent normal tissues and is associated with tumor size, differentiation degree, tumor-node-metastasis grade, lymph node metastasis, and depth of invasion in patients with colorectal cancer.<sup>11</sup> Another study reveals that Inc-TCF7 expression is higher in glioma tissues than that in normal brain tissues, and its high expression correlates with higher WHO grade (III/IV) and larger tumor size ( $\geq 3$  cm) in patients with glioma.<sup>15</sup> In addition, Inc-TCF7 is shown to activate Wnt/ $\beta$ -catenin signaling cascade, which is essential for the development of MM.<sup>13,16</sup> However, the study regarding the role of Inc-TCF7 in the pathogenesis of MM is lacking. In the present study, we investigated the correlation of Inc-TCF7 with disease risk and clinicopathological features in patients with MM. We observed that Inc-TCF7 relative expression was higher in patients with MM than that in HCs. This could be explained by that Lnc-TCF7 might facilitate the abnormal proliferation and apoptosis of monoclonal plasma cells in bone marrow through mediating Wnt signaling pathway and the mutation of extracellular signal-regulated kinase signaling pathway, which led to the malignant growth of plasma cells and increased risk of MM. Additionally, Inc-TCF7 was positively associated with ISS stage, t(4; 14), and Del(17p) chromosomal abnormality in patients with MM. The possible explanations were as follow: (a) Lnc-TCF7 was correlated with elevated severity of renal dysfunction (higher Scr and  $\beta$ 2-MG level), which contributed to advanced ISS stage in patients with MM, and (b) Lnc-TCF7 might induce aberrant class-switch recombination, V(D)J rearrangement or receptor revision via regulating its downstream pathways, which resulted in t(4; 14) and Del(17p) chromosomal abnormality in patients with MM. However, these speculations needed further validation.



**FIGURE 4** Comparison of Inc-TCF7 in MM patients with different treatment response status. The percentage of MM patients achieved CR and ORR (A). Comparison of Inc-TCF7 relative expression between MM patients who achieved CR and MM patients who did not achieve CR (B). Comparison of Inc-TCF7 relative expression between MM patients who achieved ORR and MM patients who did not achieve ORR (C). Comparison of Inc-TCF7 was performed by Wilcoxon rank sum test.  $P < .05$  was considered significant. Lnc-TCF7, long non-coding RNA T-cell factor 7; CR, complete response; ORR, overall response rate; MM, multiple myeloma



**FIGURE 5** The differences of survival profiles between MM patients with lnc-TCF7 low expression and lnc-TCF7 high expression. Comparison of PFS between MM patients with lnc-TCF7 low expression and lnc-TCF7 high expression (A). Comparison of OS between MM patients with lnc-TCF7 low expression and lnc-TCF7 high expression (B). PFS and OS were plotted with Kaplan-Meier curves, and the comparison of PFS and OS was determined by log-rank test. MM, multiple myeloma; lnc-TCF7, long non-coding RNA T-cell factor 7; PFS, progression-free survival; OS, overall survival

Existing evidence illuminates the clinical implication of lnc-TCF7 as a prognostic factor for some cancers such as colorectal cancer and glioma.<sup>14,15</sup> Colorectal cancer patients with lnc-TCF7 high expression exhibit worse overall 3-year survival rates than those with lnc-TCF7 low expression.<sup>14</sup> Another study elucidates that lnc-TCF7 is an independent predictive factor for poor OS in patients with glioma.<sup>15</sup> Considering our findings that lnc-TCF7 was associated with advanced ISS stage, t(4;14), Del(17p) chromosomal abnormality, impaired renal function (higher Scr and  $\beta$ 2-MG level), and increased LDH level in patients with MM, we hypothesized that lnc-TCF7 was associated with worse prognosis as well. In the present study, we exhibited that lnc-TCF7 was correlated with lower CR, shorter PFS, and OS in patients with MM. The possible reasons were as follow: (a) lnc-TCF7 was associated with t(4; 14) and Del(17p) chromosomal abnormalities that might trigger the increase of regulator T cells, myeloid-derived suppressor cells, plasmacytoid dendritic cells, decrease of dendritic cells, natural killer cells, B cells, T helper cells, and cytotoxic T cells in bone marrow niche, which contributed to high-risk disease states, end-organ damage, and unfavorable prognosis<sup>21</sup>; (b) lnc-TCF7 was associated with higher Scr,  $\beta$ 2-MG, LDH level, and more advanced ISS stage, which were responsible for tissue damage, end-organ injury, more accelerated disease state, and poor survival in patients with MM; and (c) lnc-TCF7 induced the aberrant activation of Wnt/ $\beta$ -catenin signaling in the bone marrow niche, which mediated the proliferation, migration, and drug resistance of MM cells, thus resulting in unsatisfied prognosis in patients with MM.<sup>23</sup> Besides, several experimental studies have illustrated the effect of lnc-TCF7 on the carcinogenesis of multiple cancers by regulating potential pathways such as miR-200c/EpCAM axis and Wnt signaling pathway,<sup>13-15,24,25</sup> while the mechanism of lnc-TCF7 underlying the pathogenesis of MM remains unclear. In glioma, lnc-TCF7 enhances the proliferation, migration, and self-renewal of glioma cells by inhibiting the miR-200c/EpCAM axis.<sup>25</sup> In hepatocellular carcinoma, lnc-TCF7 triggers Wnt signaling pathway through recruiting the Switch/Sucrose non-fermentable complex to the promoter TCF7, which subsequently facilitates self-renewal

and tumor propagation of cancer cells.<sup>13</sup> Meanwhile, the activation of Wnt signaling is reported to promote the growth, survival, migration, and invasion of MM cells.<sup>16</sup> In the light of the above context, we speculated that lnc-TCF7 might be involved in the development and progression of MM via modifying Wnt signaling as well. However, further experiments needed to verify our speculation.

This study disclosed the clinical implications of lnc-TCF7 in patient with MM, which might assist clinical decision-making and disease management of MM. However, the present study was subject to certain limitations: (a) Patients were recruited from a single center with a relatively small sample size, which might result in a selection bias and limit the generalizability of our findings. Thereby, further study with patients recruited from multiple centers needed to validate our findings. (b) The detailed mechanism of lnc-TCF7 in the development and progression of MM was not investigated. Thus, further experiments should be carried out. (c) The patient with MM included in our study were all symptomatic MM patients; thus, the predictive value of lnc-TCF7 for prognosis in asymptomatic MM patients was not explored. (d) The follow-up duration could be extended to further validate the findings. (e) Only the lnc-TCF7 expression before treatment was measured, whereas the lnc-TCF7 expression was not detected when patient with MM attained CR or relapsed, which needed further study.

In conclusion, lnc-TCF7 correlates with key exacerbated clinical features such as advanced ISS stage, t(4; 14), and Del(17p) chromosomal abnormality, as well as holds the potential as a prognostic factor for poor CR and survival profiles in patient with MM. This might offer insights for the personalized treatments, management, and prognosis improvement of patient with MM.

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#### CONFLICT OF INTEREST

None.

## ORCID

Wenfang Zhuang  <https://orcid.org/0000-0001-9951-3065>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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