

Connecting METTL3 and intratumoural CD33+ MDSCs in predicting clinical outcome in cervical cancer

Huanhe Ni

Department of Biotherapy, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou 510060, P. R. China;

<https://orcid.org/0000-0001-9875-5943>

Lin Zhang

Department of Clinical Laboratory, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, Guangdong 510060, P.R. China;

Hong-xia Chen

State Key Laboratory of Oncology in South China, Collaborative Department of Biotherapy, Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou 510060, P. R. China;

Shu-qin Dai

Department of Clinical Laboratory, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, Guangdong 510060, P.R. China;

Jiang Li (✉ lijiang2@mail.sysu.edu.cn)

State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine; Department of Biotherapy, Sun Yat-sen University Cancer Center; 651 Dongfeng East Road, Guangzhou 510060, China; <https://orcid.org/0000-0001-5596-9159>

Research

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Abstract

Background: Methyltransferase-like 3 (METTL3) is a member of the m⁶A methyltransferase family and acts as an oncogene in cancers. Recent studies suggest that host innate immunity is regulated by the enzymes controlling m⁶A epitranscriptomic changes. Here, we aim to explore the associations between the levels of METTL3 and CD33⁺ myeloid-derived suppressor cells (MDSCs) in tumour tissues and the survival of patients with cervical cancer (CC).

Methods: Paraffin tumour specimens from 197 CC patients were collected. The expression of METTL3 and CD33 was measured by immunohistochemical (IHC) staining. The clinical associations of the IHC variants were analysed by Pearson's and Spearman's chi-square tests. Overall survival (OS) and disease-free survival (DFS) were estimated by the Kaplan–Meier method and log-rank test. Hazard ratios (HRs) and independent significance were obtained via Cox proportional hazards models for multivariate analyses.

Results: We found that tumour tissues displayed increased levels of METTL3 and CD33⁺ MDSCs compared with tumour adjacent tissues from the same CC patients. Importantly, METTL3 expression was positively related to the density of CD33⁺ cells in tumour tissues ($P = 0.011$). The level of METTL3 in tumour microenvironments was significantly related to advanced tumour stages. The levels of METTL3 and CD33⁺ MDSCs in tumour tissues were notably associated with reduced DFS or OS. The Cox model analysis revealed that the level of METTL3 in tumour cells was an independent factor for patient survival, specifically for DFS (HR = 3.157, $P = 0.022$) and OS (HR = 3.271, $P = 0.012$), while CD33⁺ MDSC number was an independent predictor for DFS (HR: 3.958, $P = 0.031$). Interestingly, in patients with advanced-disease stages (II-IV), METTL3 in tumour cells was an independent factor for DFS (HR = 6.725, $P = 0.010$) and OS (HR = 5.140, $P = 0.021$), while CD33⁺ MDSC density was an independent factor for OS (HR = 8.802, $P = 0.037$).

Conclusion: Our findings suggest that CD33⁺ MDSC expansion is linked to high levels of METTL3 and that METTL3 and CD33⁺ MDSCs are independent prognostic factors in CC.

Background

Cervical cancer (CC) is one of the most common tumours, ranking fourth for both incidence and mortality in women worldwide (1-3). CC is the result of continuous infection with some strains of human papillomavirus (HPV), such as HPV16 and HPV18 (4, 5). Though there are abundant measures of prevention and cures, cervical cancer continues to exhibit high invasion and poor prognosis(6). In the past decade, researchers worldwide have found that the expression levels of molecular markers in the tumour microenvironment could be an essential factor for cervical cancer (CC) growth and metastasis (7, 8). In addition to traditional prognostic factors, including age, WHO grade, TNM stage and clinical status, some of the molecular markers could be new predictors of CC prognosis (6, 9, 10). However, there are no confirmed molecular markers for tumour progression or prognosis in CC patients. The related viral

proteins E6 and E7 have been the focal points of research for the past several years (11, 12). In other words, easily detected and significant molecular markers need to be confirmed.

Methyltransferase-like 3 (METTL3) is associated with N⁶-methyladenosine (m⁶A) RNA methylation, which is the most abundant modification ubiquitously occurring in eukaryotic mRNAs (13, 14). The m⁶A modification regulates mRNA stability or translation and can affect many functions, such as immune cell differentiation, cell development, circadian periods and tumour growth (15, 16). In previous studies, METTL3 was found to have an adverse influence on acute myeloblastic leukaemia (AML), breast cancer (BC), ovarian carcinoma, bladder cancer (BC) and gastric cancer (GC) (17-22). Additionally, m⁶A modifications are carried out by a combination of m⁶A methyltransferases (also named writers: METTL3, METTL14 and WTAP), m⁶A demethylases (also named erasers: FTO and ALKBH5) and specific RNA-binding proteins (also named readers: YTHDF1/2/3, HNRNPA2B1, IGF2BP1/2/3, eIF3 (22-24).

CD33-positive cells are usually defined as myeloid-derived suppressor cells (MDSCs) with suppressive influence on human tumour tissues (25, 26). MDSCs in the tumour environment were confirmed to be an independent indicator of poor prognosis in patients with many solid tumours (25, 27, 28). In our previous studies, the MDSC proportion was expanded in the tumour microenvironment and showed extensive negative regulatory function for anti-tumour immunity in malignancies (29-31). Recent studies have indicated that the differentiation of myeloid cells is regulated by m⁶A methyltransferases, including METTL3 (22, 32, 33). We hypothesized that MDSC expansion may be linked to the level of METTL3 in the microenvironment of CC.

In the present study, we detected the levels of METTL3 and CD33⁺ MDSCs in tumour specimens from 197 CC patients by immunohistochemical (IHC) staining. We observed an increased level of METTL3 and CD33⁺ MDSCs in tumour tissues and a positive association between the level of METTL3 and CD33⁺ MDSCs. The high levels of METTL3 and CD33⁺ MDSCs in CC microenvironments were significantly associated with poor disease-free survival (DFS) and overall survival (OS) in CC patients. Importantly, METTL3 and CD33⁺ MDSCs were independent prognostic predictors for CC patients. These findings suggest that METTL3 and MDSCs contribute to the development of disease and that METTL3 may respond to MDSC expansion in tumour microenvironments in CC.

Methods

Patients and tissue samples

A total of one hundred ninety-seven CC patients who received therapy at Sun Yat-Sen University Cancer Center in Guangzhou, China, and accepted medical follow-up continued until 2019 were included. Paraffin tumour specimens from 197 CC patients were collected at Sun Yat-Sen University Cancer Center between 2008 and 2010. In this retrospective study, none of the patients received anti-tumour treatment before surgery, and all 197 patients were histologically confirmed as having primary CC.

The detailed clinical characteristics of the patients are shown in Table 1. The median age of all patients was 44 years (range 28-79). Regarding tumour grade, 16 (8.1%) patients had G1, 107 (54.3%) had G2, 66 (33.5%) had G3 and 8 (4.1%) had G4 tumours. According to the International Union against Cancer TNM staging system, 147 (74.6%) patients were in stage T1 and 50 (25.4%) patients were in stage T2-T4, while 173 (87.8%) patients were in stage N0, 24 (12.2%) patients were in stage N1, and no patients had metastasis in this study. According to the WHO classification criteria, 127 (64.5%) patients had stage I disease and 70 (35.5%) had stage II-IV disease; 26 (13.2%) died. Forty-five (22.8%) patients received surgery alone, 41 (20.8%) patients received radiation therapy alone, 27 (13.7%) patients received surgery & radiation therapy and 84 (42.6%) patients received surgery & radiation therapy & chemotherapy. The tumour specimens and clinical information were provided by the Pathology Department of Sun Yat-Sen University Cancer Center. For a total of 197 patients, the 120-month DFS rate was 11.17% and the 120-month OS rate was 13.20% (Figure S1 A and B). The study was approved by the Research Ethics Committee of the Sun Yat-sen University Cancer Center, and we obtained written informed consent from all 197 patients.

Immunohistochemistry

We used an immunohistochemistry kit (ZSGB Bio, Beijing, China) for IHC staining according to the manufacturer's instructions. The expression level was scored for METTL3 in tumour cells and tumour-infiltrating immune cells in five separate ×400 high-power fields (HPFs). We scored METTL3 expression in the tumour cells and tumour-infiltrating immune cells of each specimen by using a semiquantitative immunoreactivity scoring system, which ranged from zero to twelve and was equal to multiplication of the intensity of immunohistochemical staining (zero: no staining; one: weak staining; two: moderate staining; and three: strong staining) and the percentage of positive tumour cells (one: less than 25%; two: 25–50%; three: 50–75%; and four: more than 75%). The expression of CD33 was evaluated based on the mean percentage from five separate ×400 HPFs from the same patient, in which we counted the CD33-positive cells and tumour-infiltrating immune cells and then divided the number of CD33-positive cells by the number of tumour-infiltrating immune cells. These METTL3- and CD33-positive scores were determined separately by two pathologists, and their averages were taken. The isotype antibody was included as a control.

Antibodies and Reagents

The antibodies used in this research were as follows: rabbit anti-METTL3 antibody (ab195352; Abcam, USA, 1:400), rabbit anti-CD33 antibody (ab199432; Abcam, USA, 1:200), and rabbit mAb IgG control (ab172730; Abcam, USA, 1:200). An immunohistochemistry kit (PV-6001-6.0; ZSGB Bio, Beijing, China) was also used.

Statistics

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used to analyse all the data, and GraphPad Prism 7 software (La Jolla, CA, USA) was used to obtain the curves. The median values were used as cutoff

values to divide the patients into two groups (high level and low level). We used Pearson's chi-square test or Spearman's chi-square test to analyse the relationships between immunohistochemical variants in different cell populations and patients' clinical parameters. The relationships among the expression of METTL3 in tumour cells, METTL3 in tumour-infiltrating immune cells and CD33 in tumour-infiltrating immune cells were determined using Pearson's or Spearman's correlation coefficient and linear regression analyses. Then, we analysed prognostic factors in univariate and multivariate analyses by using the Cox proportional hazards model. In our research, $*P < 0.05$ was considered significant.

Results

The level of METTL3 is positively linked to the number of CD33⁺ MDSCs and contributes to tumour development

In the present study, the levels of METTL3 and CD33⁺ MDSCs were examined in tumour specimens from 197 patients with CC by IHC. METTL3 was located in the nucleus of tumour cells and tumour-infiltrating immune cells (Figure 1 A and B), while CD33⁺ cells were scattered mainly in the tumour stroma (Figure 1 C and D), and isotype IgG was used as a control (Figure 1 E). The levels of METTL3 and CD33⁺ MDSCs in tumour tissues were 63.45% (125/197) and 52.28% (103/197), respectively (Figure 1). Among the 197 patients with CC, the median survival time was 96 months (range: 0 to 120 months); the 10-year DFS rate and 10-year OS rate were 88.83% and 86.80%, respectively (Figure S1 A and B). Table 2 shows the results of the relationships between clinicopathological features and immunohistochemical variants in different cell types in the tumour microenvironment. High METTL3 expression in the tumour and in tumour-infiltrating immune cells was linked to advanced tumour stage ($P = 0.04$ and 0.02 , respectively).

In addition, we analysed the relationship between METTL3 expression in tumour cells and in tumour-infiltrating immune cells and the number of CD33⁺ MDSCs via Spearman's correlation coefficient and linear regression. The expression of METTL3 in tumour cells was positively correlated with that in tumour-infiltrating immune cells ($R = 0.264$, $P < 0.001$) (Figure 2 A). The number of CD33⁺ cells was positively correlated with the expression of METTL3 in tumour cells ($R = 0.145$, $P = 0.041$) and tumour-infiltrating immune cells ($R = 0.182$, $P = 0.011$) (Figure 2 B and C).

High METTL3 levels and CD33⁺ MDSC density are associated with poor outcomes

To evaluate the expression of METTL3 and CD33 as predictors for the prognosis of the 197 patients, Kaplan–Meier survival curves were used for analysis. The high level of METTL3 in tumour cells was significantly correlated with decreased DFS ($P < 0.001$, Figure 3A) and OS ($P < 0.001$, Figure 3B) in CC patients. Accordingly, a high level of METTL3 in tumour-infiltrating immune cells was negatively correlated with DFS ($P = 0.002$, Figure 3C) and OS ($P < 0.001$, Figure 3D) in CC patients. The high density of CD33⁺ MDSCs was obviously correlated with decreased DFS ($P < 0.001$, Figure 3E) and OS ($P < 0.001$, Figure 3F) in CC patients.

METTL3 and CD33⁺ MDSCs are independent factors for patient prognosis

Univariate analysis showed that in addition to lymph node and clinical stage, high levels of METTL3 in tumour cells (HR: 4.244, $P = 0.002$) and in tumour-infiltrating immune cells (HR: 4.857, $P = 0.004$) and a high density of CD33⁺ MDSCs (HR: 6.579, $P = 0.002$) were noticeably correlated with reduced DFS. In addition, we also found that high levels of METTL3 in tumour cells (HR: 5.502, $P = 0.001$) and in tumour-infiltrating immune cells (HR: 6.021, $P = 0.001$) and a high density of CD33⁺ MDSCs cells (HR: 5.755, $P = 0.001$) were associated with decreased OS. As shown in Table 3, clinicopathological parameters such as clinical stage (HR: 3.511, $P = 0.005$) and nodal status (HR: 2.798, $P = 0.032$) also had prognostic value with decreased DFS, and clinical stage (HR: 3.820, $P = 0.001$) was related to decreased OS. In addition, other clinical characteristics, such as age and tumour status, were not clearly related to DFS and OS (Table 3). When we performed the multivariate Cox proportional hazards regression analysis, we included all the significant univariate variables. For all 197 patients, in addition to clinical stage (HR: 3.827, $P = 0.003$), N status (HR: 3.219, $P = 0.021$) was an independent factor for DFS, clinical stage (HR: 4.248, $P < 0.001$) was an independent factor for OS, and METTL3 levels in tumour cells (HR: 3.157, $P = 0.022$) and in tumour-infiltrating immune cells (HR: 3.368, $P = 0.036$) and CD33⁺ MDSCs (HR: 3.958, $P = 0.031$) were independent factors for both DFS and OS (Table 4).

METTL3 and CD33⁺ MDSCs have predictive value for patients with early and advanced disease stages

We further divided the 197 patients into two subgroups based on the clinicopathological stage: 127 of the total patients were in early disease stage (stage I), while 70 of the total patients were in advanced disease stage (stage II-IV). Through Kaplan–Meier method, we found that the high expression of METTL3 in tumour-infiltrating immune cells was significantly correlated with poor DFS ($P = 0.033$) and OS ($P = 0.019$) (Figure S2 C and D) in patients with early disease stage, while there was no significant association between the high expression of METTL3 in tumour cells ($P = 0.400$ vs $P = 0.183$) and the number of CD33⁺ MDSCs ($P = 0.393$ vs $P = 0.227$) with the DFS and OS of patients with early-stage disease (Figure S2 A, B, E and F). For patients with advanced-stage disease ($n = 70$), a high level of METTL3 in tumour cells was dramatically correlated with decreased DFS ($P < 0.001$, Figure 4 A) and OS ($P < 0.001$, Figure 4B), and a high level of METTL3 in tumour-infiltrating immune cells was negatively correlated with DFS ($P = 0.004$, Figure 4 C) and OS ($P < 0.001$, Figure 4D); the increased number of CD33⁺ MDSCs was dramatically correlated with poor DFS ($P < 0.001$, Figure 4 E) and OS ($P < 0.001$, Figure 4F). Using multivariate Cox regression analysis in the 70 patients with advanced-stage disease, METTL3 expression in tumour cells (HR: 6.725, $P = 0.010$) was an independent prognostic factor for DFS, while METTL3 expression in tumour cells (HR: 5.140, $P = 0.021$) and CD33⁺ MDSCs (HR: 8.802, $P = 0.037$) were independent prognostic factors for OS (Table 4).

The combination of METTL3 levels and CD33⁺ MDSCs was associated with the survival of patients with CC

Finally, considering that METTL3 levels were positively correlated with high CD33⁺ MDSC infiltration, we calculated significance of the combination of these two biomarkers for the survival of CC patients. All 197 patients were divided into three groups. Patients with low levels of both METTL3 in tumour-infiltrating immune cells and CD33⁺ MDSCs were included in the combined low expression group, those with only one of the two biomarkers with high levels were included in the combined medium expression group, and those with high levels of both were included in the combined high expression group. The high combination of METTL3 and intratumoural CD33⁺ MDSCs was associated with reduced DFS ($P < 0.001$, Figure 5A) and OS ($P < 0.001$, Figure 5B). In the patients (127) with early-stage disease, the high combination of METTL3 and CD33⁺ MDSCs was not related to DFS ($P = 0.063$, Figure 5C) but was clearly negatively related to OS ($P = 0.037$, Figure 5D). In the patients (70) with advanced-stage disease, the high combination of METTL3 in and CD33⁺ MDSCs was clearly related to unfavourable DFS ($P < 0.001$, Figure 5E) and OS ($P < 0.001$, Figure 5F).

Indeed, compared to METTL3 or CD33⁺ MDSCs, the combination of METTL3 and CD33⁺ MDSCs can improve the prognostic stratification of survival for CC patients, especially those in advanced disease stages. Based on a total of 197 patients, the high combination of METTL3 levels and CD33⁺ MDSCs was a predictor of worse patient prognosis, including DFS [HR (95% CI): 4.672 (2.149-10.156), $P < 0.001$] and OS [HR (95% CI): 4.890 (2.369-10.093), $P < 0.001$]. In the patients with early-stage disease, we found that the high combination of METTL3 levels and intratumoural CD33⁺ MDSCs was negatively correlated with OS [HR (95% CI): 3.071 (1.056-8.931), $P = 0.039$], but there was no significant association with DFS. Importantly, we found that the high combination of tumour METTL3 and intratumoural CD33⁺ MDSCs was significantly correlated with poor DFS [HR (95% CI): 7.673 (2.420-24.324), $P = 0.001$] and OS [HR (95% CI): 7.286 (2.667-19.902), $P < 0.001$] in patients with advanced disease stages (Table 3), suggesting that the high combination of METTL3 levels and CD33⁺ MDSCs improved patient prognostic stratification in those with advanced-disease.

Discussion

The development of tumour cells depends on the tumour microenvironment, which includes tumour cells, various other cells and extracellular components(7). The immunosuppressive cells in the tumour microenvironment, such as Tregs and MDSCs, not only affect each other, but their changes in number and types will affect tumour development (34, 35). METTL3 is one of the Writers, and its role is to catalyse the m⁶A methylation of mRNA (and other nuclear RNAs); after the methylation of m⁶A, RNAs will nucleate and transport to the cytoplasm faster and then produce more proteins for function and proliferation. Some studies have shown that METTL3 expression can promote tumour cell proliferation, leading to poor patient prognosis. The tumour-infiltrated MDSC population usually induces anti-tumour immunity tolerance by inhibiting the proliferation and function of T cells, such as hindering antigen presentation by antigen-presenting cells (36). Increased METTL3 and CD33⁺ MDSCs have been found in tumour microenvironments and lead to a poor prognosis (37-40). In this study, we focused on the

distribution of METTL3 and CD33⁺ MDSCs in the tumour microenvironment of 197 patients with CC. The positive association between METTL3 levels and CD33⁺ MDSCs and the prognostic value of these two variants in CC patients were demonstrated.

M⁶A methyltransferases, especially METTL3, can affect many physiological and pathological diseases through *p53* and other genes (14). At the nucleic acid level, silencing m⁶A methyltransferase significantly affects gene expression and mRNA splicing patterns, leading to changes in normal cell signaling pathways and apoptosis (33). In bladder cancer cells, m⁶A-modified direct targets IKBKB and RELA (two key regulators of the NF-κB pathway) mediated by METTL3 become factors that promote tumour development (13); in glioblastoma stem cells (GSCs), knocking down METTL3 can induce changes in m⁶A-enriched mRNA and alter the mRNA expression of genes with key biological functions in GSCs (such as ADAM1937) (41). In recent studies, high METTL3 expression levels were related to tumour invasion and poor outcomes in breast cancer and acute myeloid leukaemia (AML) (21, 42). Our results are consistent with the results of these studies, showing that high METTL3 expression results in poor prognosis in CC patients. METTL3 regulates haematopoietic stem cell differentiation and induces the development of leukaemic cells by upregulating MYC expression (42, 43). Therefore, we wondered whether METTL3 expression may be linked to the density of tumour-infiltrated MDSCs. Our data for the first time identified a positive association between METTL3 expression in tumour cells and in tumour-infiltrating immune cells and intratumoural CD33⁺ MDSC density in CC. Moreover, both METTL3 and CD33⁺ MDSCs were independent factors for the prognosis of CC patients, and the combination of METTL3 levels and CD33⁺ MDSC density displayed prognostic value for CC patients, including patients at early or late disease stages. However, further investigation is currently underway to identify additional mechanisms by which METTL3 may be involved in the regulation of tumour-infiltrated CD33⁺ MDSC expansion in CC. The function, distribution and clinical relevance of the proportion of tumour-derived CD33⁺ MDSCs have been explored in recent years. MDSCs are generally elevated in tumour tissues and in the peripheral blood of cancer patients and are linked to anti-tumour immunity suppression, resulting in tumour growth and metastasis (25, 34, 44). In our study, CC patients with a high infiltration of MDSCs in the cervical cancer microenvironment showed a poor prognosis, which is consistent with observations in other solid cancers.

The tumour microenvironment is a main battle ground between tumour cells and the host immune system. Tumour cells usually 'educate' infiltrated immune cells through many factors, such as cytokines or tumour-derived exosomes, to affect the proliferation, differentiation and function of tumour-infiltrating immune cells, resulting in the expansion of suppressive immune cells, including M2 macrophages, MDSCs and Tregs, and limiting the anti-tumour effect of cytotoxic T cells. Epigenetic modifications, such as RNA modification, DNA methylation and histone modifications, can rapidly regulate infiltrated immune cell differentiation and activities in tumour microenvironments (45). Here, our data suggest that METTL3-mediated m⁶A RNA modification may be involved in the regulation of infiltrated MDSC induction in tumour microenvironments and affect tumour development and prognosis through infiltrated MDSC levels in CC. We further demonstrate for the first time the prognostic value of the combination of the

METTL3 level in tumour-infiltrating immune cells and CD33⁺ MDSC density in CC patients, especially for patients in advanced disease stages. However, a mechanistic study to support the role of METTL3 in the regulation of tumour-derived MDSC differentiation is currently underway and the underlying mechanisms will be clarified in the near future.

Conclusions

This is the first time that comprehensive results of relationship between METTL3 and CD33 have been reported in CC patients by using IHC. Results have been shown that both biomarkers were adverse indicators for prognosis and may have the significant relationship in microenvironment of CC. Moreover, the combined expressions of METTL3 and CD33 in tumor-infiltrating immune cells were observably positively correlated. Our research may give cues for further researches on that the mechanism of METTL3 and CD33 in CC microenvironment.

List Of Abbreviations

METTL3, Methyltransferase-like 3; CC: Cervical cancer; MDSC: Myeloid-derived suppressor cells; DFS: Disease-free survival; OS: Overall survival; TNM: Tumornode-metastasis; WHO: World Health Organization; T stage: Tumor status; N status: Lymph node metastasis.

Declarations

Ethics approval and consent to participate

Total research procedures were performed with the approval of Research Ethics Committee of the Sun Yat-sen University Cancer Center (GZR2013-040).

Consent for publication

The authors declare no conflicts of interest. All authors read and approved the final manuscript.

Availability of data and materials

The authenticity of this article has been validated by uploading the key raw data to the Research Data Deposit (RDD) public platform (www.researchdata.org.cn).

Competing interests

The authors have declared that they have no competing interest. The sources that funded this study played no role in the study design, data collection, data analysis, decision to publish, or preparation of the manuscript.

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Authors' contributions

Conceived and designed the experiments: JL. Performed the experiments: HHN, LZ. HXC. Analyzed the data: JL, HHN, LZ. Contributed reagents/materials/analysis tools: SQD. Wrote the manuscript: JL, HHN, LZ. All authors read and approved the final manuscript.

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Tables

Table 1 Clinical characteristics of total 197 patients with cervical cancer.

Characteristics	No. of patients (%)
Total case	197
Age (Years)	
Median, Range	44, 28-79
44	106 (53.8%)
44	91 (46.2%)
Gender	
Female	0 (0%)
Male	197(100.0%)
Tumor Grade ^a	
1	16 (8.1%)
2	107 (54.3%)
3	66 (33.5%)
4	8 (4.1%)
Pathological Tumor (T) Status ^b	
1	147 (74.6%)
2-4	50 (25.4%)
Pathological Node (N) Status ^b	
0	173 (87.8%)
1	24 (12.2%)
Pathological metastasis (M) Status ^b	
0	197 (100.0%)
1	0 (0%)
Clinical stage ^c	
I-IV	127 (64.5%)
V	70 (35.5%)
Treatment	
Observation	171 (86.8%)
Surgery	26 (13.2%)
Therapy after surgery	
Surgery alone	45 (22.8%)
Radiation therapy alone	41 (20.8%)
Surgery & Radiation therapy	27 (13.7%)
Surgery & Radiation therapy & Chemotherapy	84 (42.6%)

a, Tumor Grade is according to WHO classification criteria

b, Pathological Tumor (T) Status, Pathological Node (N) Status and Pathological metastasis (M) Status are from International Union against Cancer 2002 TNM staging system

c, Clinical stage is according to International Federation of Gynecology and Obstetrics (FIGO).

Table 2 Correlations the expression of METTL3, CD33 and clinical parameters in total 197 cervical cancer patients.

Clinicopathologic parameter	Total case	High METTL3 levels in tumor (%) (%)	<i>P</i>	High METTL3 levels in tumor-infiltrating immune cells (%)	<i>P</i>	High density of CD33 ⁺ MDSCs (%)	<i>P</i>
Age							
≤44 (y)	86	31 (43.7%)	0.999 ^a	41 (47.7%)	0.524 ^a	46 (53.5%)	0.672 ^a
>44 (y)	111	40 (56.3%)		58 (52.3%)		56 (50.5%)	
WHO grade							
G1	16	6 (37.5%)	0.835 ^b	8 (50.0%)	0.075 ^a	5 (31.3%)	0.173 ^a
G2	107	37 (34.6%)		51 (47.7%)		53 (49.5%)	
G3	66	26 (39.4%)		39 (59.1%)		40 (60.6%)	
G4	8	2 (25.0%)		1 (12.5%)		4 (50.0%)	
T status							
T1	147	59 (40.1%)	0.040 ^a	81 (55.1%)	0.020 ^a	78 (53.1%)	0.536 ^a
T2-4	50	12 (24.0%)		18 (36.0%)		24 (48.0%)	
N status							
N0	173	64 (37.0%)	0.454 ^a	88 (50.9%)	0.644 ^a	88 (50.9%)	0.493 ^a
N1	24	7 (29.2%)		11 (45.8%)		14 (58.3%)	
Clinical stage							
I	127	47 (37.0%)	0.703 ^a	68 (53.5%)	0.214 ^a	63 (49.6%)	0.412 ^a
II-IV	70	24 (34.3%)		31 (44.3%)		39 (55.7%)	

a, Data are analyzed by Pearson's chi-squared test.

b, Data are analyzed by Spearman's chi-squared test.

Table 3 The univariate cox regression analysis in cervical cancer patients.

Factors	Disease-free survival		Overall survival	
	HR (95%CI)	P value	HR (95%CI)	P value
The levels of METTL3, CD33 in tumor and tumor-infiltrating immune cells (n = 197)				
Age, years ($\leq 44 / > 44$)	0.937 (0.405-2.169)	0.879	1.281 (0.581-2.825)	0.539
Clinical stage (I/II-IV)	3.511 (1.472-8.373)	0.005	3.820 (1.702-8.573)	0.001
Tumor (T) status (1/2-4)	0.128 (0.017-0.955)	0.051	0.106 (0.014-0.783)	0.058
Nodal (N) status (0/1)	2.798 (1.095-7.152)	0.032	2.225 (0.893-5.542)	0.086
METTL3 in tumor cells (low/high)	4.244 (1.730-10.413)	0.002	5.502 (2.312-13.092)	0.001
METTL3 in tumor-infiltrating immune cells (low/high)	4.857 (1.643-14.353)	0.004	6.021 (2.074-17.474)	0.001
Number of CD33 ⁺ MDSCs (low/high)	6.579 (1.946-22.241)	0.002	5.755 (1.982-16.705)	0.001
Combination of METTL3 levels and CD33 ⁺ MDSCs (low/ middle/ high)	4.672 (2.149-10.156)	<0.001	4.890 (2.369-10.093)	<0.001
The levels of METTL3, CD33 in tumor and tumor-infiltrating immune cells in stage I (n = 127)				
Age, years ($\leq 44 / > 44$)	0.364 (0.074-1.807)	0.217	0.562 (0.140-2.250)	0.416
Tumor (T) status (1/2-4)	0.033 (0.00-35.599)	0.338	0.032 (0.000-22.782)	0.305
Nodal (N) status (0/1)	2.736 (0.552-13.569)	0.218	2.325 (0.483-11.20)	0.293
METTL3 in tumor cells (low/high)	1.696 (0.424-6.783)	0.455	2.184 (0.586-8.132)	0.244
METTL3 in tumor-infiltrating immune cells (low/high)	6.489 (0.799-52.832)	0.080	7.452 (0.932-59.588)	0.058
Number of CD33 ⁺ MDSCs (low/high)	1.732 (0.414-7.250)	0.452	2.085 (0.521-8.339)	0.299
Combination of METTL3 levels and CD33 ⁺ MDSCs (low/ middle/ high)	2.639 (0.896-7.775)	0.078	3.071 (1.056-8.931)	0.039
The levels of METTL3, CD33 in tumor and tumor-infiltrating immune cells in stage II-IV (n = 70)				
Age, years ($\leq 44 / > 44$)	0.953 (0.299-3.039)	0.935	1.240 (0.404-3.804)	0.707
Tumor (T) status (1/2-4)	0.145 (0.019-1.110)	0.063	0.116 (0.015-0.874)	0.537
Nodal (N) status (0/1)	2.554 (0.800-8.152)	0.113	1.192 (0.623-5.869)	0.257
METTL3 in tumor cells (low/high)	9.713 (2.697-34.978)	0.001	13.199 (3.776-46.130)	0.001
METTL3 in tumor-infiltrating immune cells (low/high)	5.373 (1.498-19.276)	0.010	7.209 (2.070-25.106)	0.002
Number of CD33 ⁺ MDSCs (low/high)	66.197 (0.891-4917.536)	0.056	16.621 (2.199-125.660)	0.006
Combination of METTL3 levels and CD33 ⁺ MDSCs (low/ middle/ high)	7.673 (2.420-24.324)	0.001	7.286 (2.667-19.902)	<0.001

HR = hazard ratio,

95%CI = 95% confidence interval.

*Since combined expression of METTL3 and CD33 in tumor-infiltrating immune cells is divided into three groups (low/ middle/ high), it is no longer calculated in multivariate analysis.

Table 4 The multivariate cox regression analysis in cervical cancer patients.

Factors	Disease-free survival		Overall survival	
	HR (95%CI)	P value	HR (95%CI)	P value
Low levels of METTL3, CD33 in tumor and tumor-infiltrating immune cells (n = 197)				
clinical stage [I/II-IV]	3.827 [1.585-9.241]	0.003	4.248 (1.886-9.571)	<0.001
tumor (T) status (1/2-4)	-	-	--	--
nodal (N) status (0/1)	3.219 [1.197-8.653]	0.021	--	--
METTL3 in tumor cells (low/high)	3.157 [1.181-8.438]	0.022	3.271 (1.303-8.213)	0.012
METTL3 in tumor-infiltrating immune cells (low/high)	3.368 [1.080-10.502]	0.036	1.006 (0.202-5.013)	0.994
number of CD33 ⁺ MDSCs (low/high)	3.958 (1.138-13.767)	0.031	--	--
Low levels of METTL3, CD33 in tumor and tumor-infiltrating immune cells in stage II-IV (n = 70)				
tumor (T) status (1/2-4)	--	--	0.173 [0.023-1.317]	0.090
METTL3 in tumor cells (low/high)	6.725 [1.576-28.696]	0.010	5.140 [1.286-20.548]	0.021
METTL3 in tumor-infiltrating immune cells (low/high)	2.053 [0.483-8.726]	0.330	2.149 [0.541-8.540]	0.277
number of CD33 ⁺ MDSCs (low/high)	-	-	8.802 [1.136-68.233]	0.037

The significant different factors in univariate analysis were analyzed by multivariate analysis, and the factors which were not significant in univariate analysis were not included.

Supplemental Figure Legends

Figure S1

The DFS curve and OS curve of 197 CC patients, and expression of METTL3 and CD33 in tumours or in tumour-adjacent tissues.

The disease-free survival (DFS) curve and overall survival (OS) curve of 197 CC patients in this study (A and B). Statistical analysis revealed that the expression of METTL3 and the number of CD33⁺ MDSCs were not significantly different between tumour-adjacent tissues (Non-tumour) and tumour tissues (Tumour) (C and D).

Figure S2

Kaplan–Meier curves of DFS and OS according to METTL3 expression in different cell populations and intratumoural CD33⁺ cells of early-stage patients.

In 127 patients with early-stage CC, no association was found between DFS or OS and the METTL3 expression in tumour cells (A and B). DFS and OS associations with METTL3 expression in tumour-infiltrating immune cells (C and D). No association was found between the DFS or OS and intratumoural CD33⁺ MDSCs (E and F).

Figures

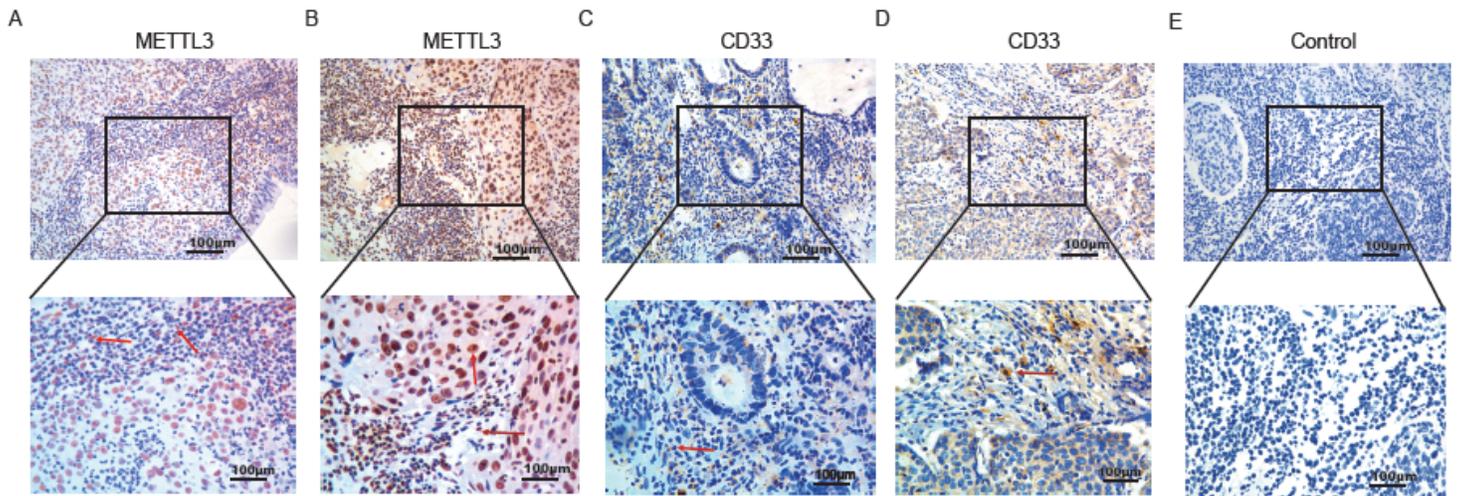


Figure 1

The expression of METTL3 and CD33 in cervical cancer METTL3 (A and B) and CD33 (C and D) immunohistochemical staining in representative examples of cervical cancer. METTL3 staining was observed in both tumour cells and tumour-infiltrating immune cells. The negative control of the tissue (E). The upper panel is $\times 200$ original magnification, and the lower panel is $\times 400$ original magnification. The positive sites in tumour and tumour-infiltrating immune cells are indicated by the red arrow and black arrows, respectively.

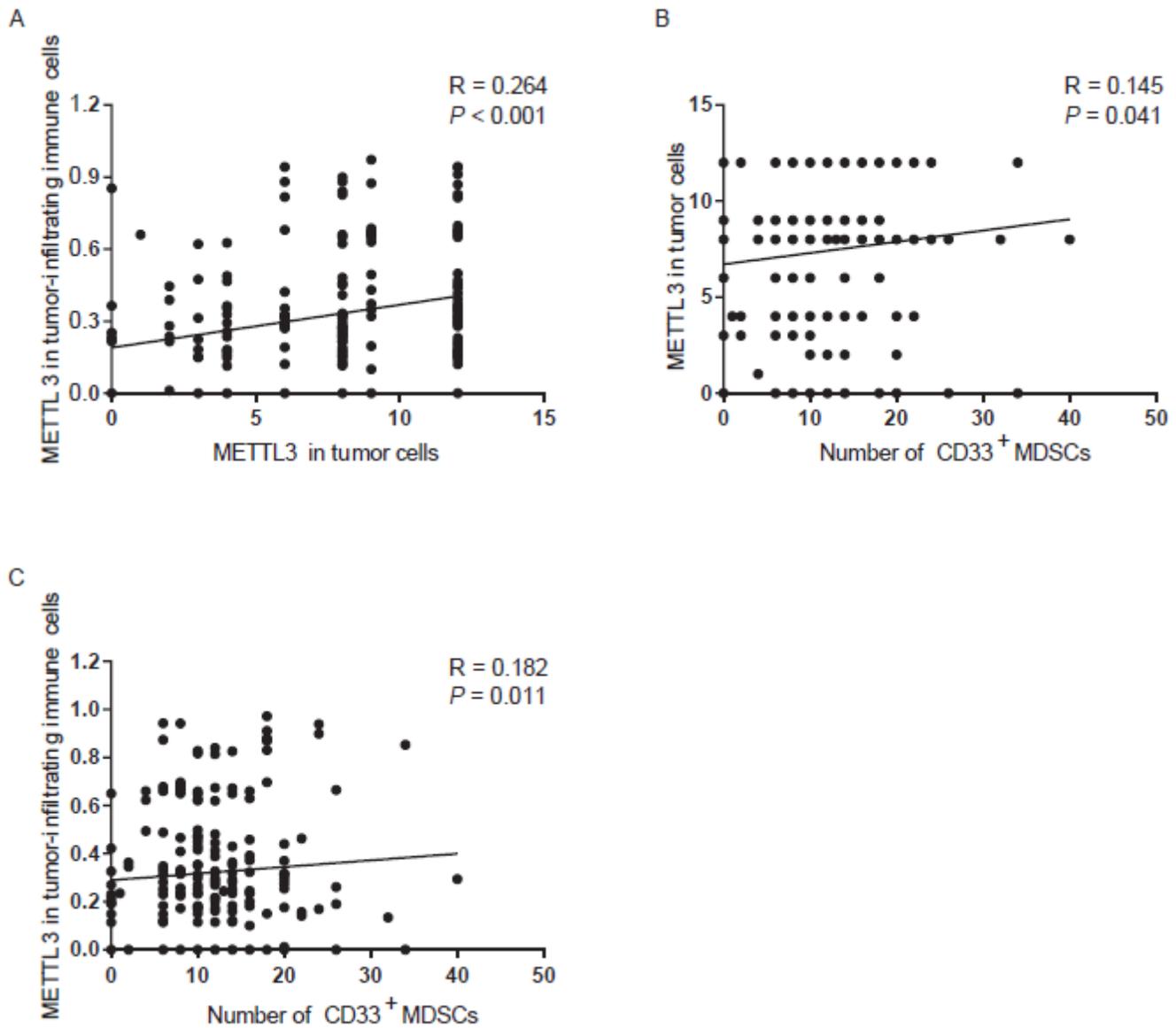


Figure 2

Tumor METTL3 level was positively related to intratumoural CD33⁺ MDSCs. METTL3 expression in tumour cells was observably positively correlated with the expression of METTL3 in tumour-infiltrating immune cells (A). Correlation between intratumoural CD33⁺ MDSCs and METTL3 expression in tumour (B) or tumour-infiltrating immune cells (C).

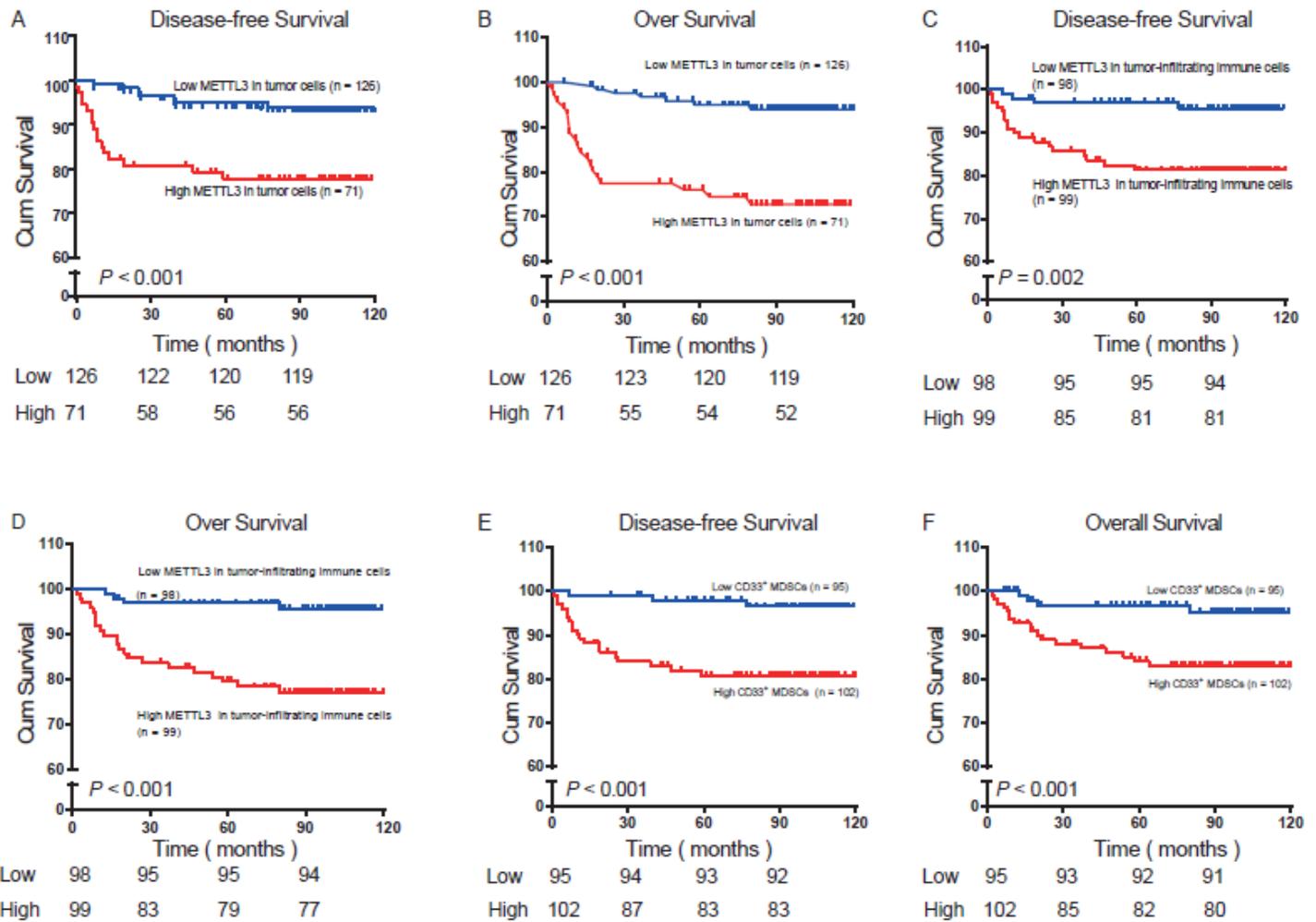


Figure 3

Kaplan–Meier curves of DFS and OS according to METTL3 level or intratumoural CD33+ MDSCs in 197 CC patients. The relationship of DFS and OS with METTL3 expression in tumour (A and B) and tumour-infiltrating immune cells (C and D). The relationship of DFS and OS with intratumoural CD33+ MDSCs in the immune stroma (E and F). The percentage of DFS and OS was calculated by the Kaplan–Meier method and P values were calculated by the log-rank statistic.

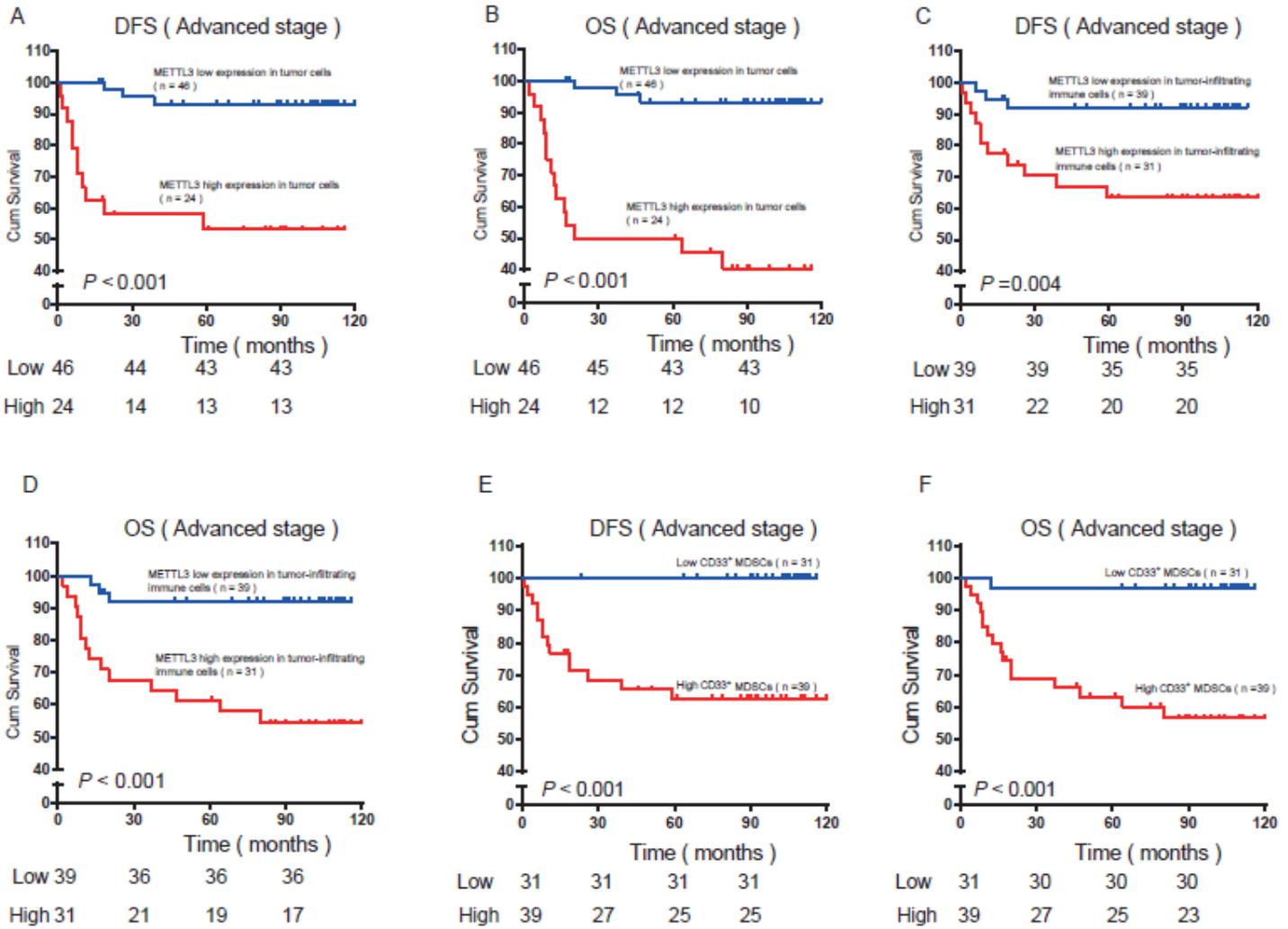


Figure 4

Kaplan–Meier curves of DFS and OS according to METTL3 level and intratumoural CD33+ MDSCs in CC patients with advanced stage disease. In 70 patients with advanced-stage CC, the relationship of DFS and OS with METTL3 expression in tumour cells (A and B). The relationship of DFS and OS with METTL3 expression in tumour-infiltrating immune cells (C and D). The intratumoural CD33+ MDSCs were related to poor DFS and OS (E and F).

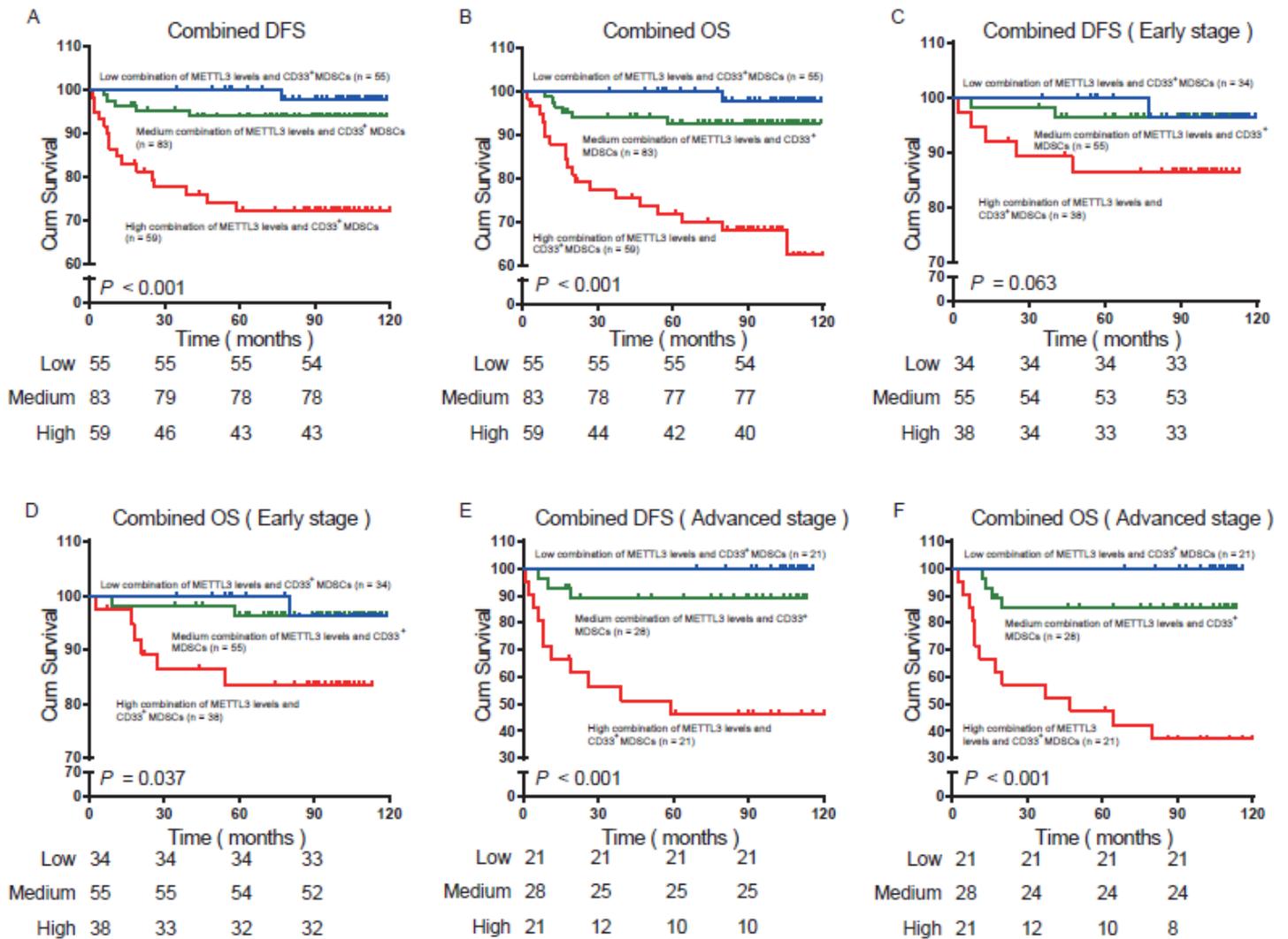


Figure 5

Kaplan–Meier curves of DFS and OS according to the combination of METTL3 levels and CD33+ MDSCs in CC patients with early or advanced stage disease. In a total of 197 patients, DFS and OS were negatively related to the combination of METTL3 levels and CD33+ MDSCs (A and B). In 127 early-stage patients, DFS was negatively related to the combination of METTL3 levels and CD33+ MDSCs, but no association was found between the OS and the combination of METTL3 levels and CD33+ MDSCs (C and D). In 70 patients with advanced-stage CC, DFS and OS were negatively related to the combination of METTL3 levels and CD33+ MDSCs (E and F).

Supplementary Files

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