Prognostic significance of tumor-associated neutrophils and circulating neutrophils in glioblastoma, IDH wild-type

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Research Article

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Abstract

Background

Tumor-associated neutrophils (TANs) in the tumor microenvironment are prognostic biomarkers in many malignancies. However, the prognostic value of TANs in glioblastoma, IDH wild-type remains poorly studied. We analyzed correlations of TANs and peripheral blood neutrophils prior to radiotherapy with overall survival (OS) in glioblastoma.

Methods

RNA-seq expression profiles of patients with newly diagnosed glioblastoma, IDH wild-type were extracted from The Cancer Genome Atlas (TCGA), and The Chinese Glioma Genome Atlas (CGGA). TAN infiltration was inferred using CIBERSORTx algorithm. Neutrophil counts prior to radiotherapy in newly diagnosed glioblastoma, IDH wild-type were obtained from the First Affiliated Hospital of Fujian Medical University. The prognostic value of TANs and peripheral blood neutrophils before radiotherapy was investigated using Kaplan-Meier analysis and Cox proportional hazards models. The robustness of these findings was evaluated by sensitivity analysis, and E values were calculated.

Results

A total of 113 and 173 individuals with glioblastoma, IDH wild-type were identified from the TCGA and CGGA cohorts, respectively. High infiltration of TANs was of prognostic of poor OS in TCGA (HR = 1.811,95% CI: 1.048–3.128) and CGGA (HR = 1.572, 95% CI: 1.046–2.365). Levels of peripheral blood neutrophils before radiotherapy (HR = 2.073, 95% CI: 1.077–3.990) were independently associated with poor prognosis. Sensitivity analysis determined that the E-value of high TANs infiltration was 2.38 and 2.52 in the TCGA and CGGA cohorts.

Conclusions

TANs and peripheral blood neutrophil levels before radiotherapy are prognostic of poor outcomes in glioblastoma, IDH wild-type.

Introduction

Glioblastoma is a highly malignant type of glioma, and accounts for over 50% of emerging glioma cases [1]. Treatment with postoperative chemoradiotherapy is currently the standard of care for glioblastoma. Even though some patients may benefit from the Stupp regimen [2], the overall prognosis remains poor [3], with less than 10% survivorship at 5 years [4]. At present, prognostic predictions for patients with glioblastoma are mainly made based on patient age, Karnofsky Performance Status (KPS), prior

treatment, resection range, methylation of O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status, isocitrate dehydrogenase genes (IDH), telomerase reverse transcriptase (TERT), and other molecular markers, such as alpha thalassemia/mental retardation syndrome X-linked (ATRX) gene [5, 6]. It is of paramount significance to make overall assessment of patients, and to evaluate potential high-risk factors that affect patient prognosis, to improve the timely adjustment of treatment methods and the accuracy of prognostic assessments.

There are different driver genes, molecular characteristics, and clinical prognosis associated with either IDH mutant or IDH wild-type glioblastoma [7]. Glioblastoma, IDH wild-type is considered as an independent genotyping for diagnosis based on the fifth edition of the 2021 World Health Organization classification of tumors of the central nervous system[1], thus further advancing the role of molecular neuropathology in CNS tumor classification. Compared with the IDH mutant type, glioblastoma patients with IDH wild type have higher invasiveness, and poorest prognosis, with a median survival time ranging from 6 to 15 months [8]. As studies have shown, 30% to 50% of IDH wild-type glioblastoma demonstrates methylation of the MGMT promoter, which is associated with favorable clinical responses to TMZ, and is considered to be a poor prognostic factor [9]. However, the utility of this biomarker may be limited by acquired drug resistance, and disease prognosis still varies greatly. Therefore, among the glioblastoma, IDH wild-type population, there still must be additional reliable biomarkers developed for patient stratification and disease prognosis.

During the progression of glioblastoma, factors such as the tumor microenvironment, and infiltration of non-tumor cells and immune cells influences the gene expression and transcription types of glioblastoma [10], and can result in the interconversion of molecular subtypes. Neutrophils are important members of the tumor microenvironment. Neutrophils exhibit tumor-promoting activity by inducing angiogenesis [11-14], inhibiting T cell activation (immunosuppression) [15-19], inducing genetic instability [20-22], and maintaining tumor cell proliferation [23-26]. Tumor-associated neutrophils (TANs) are also prognostic markers for patients with tumors [27-29], and are closely related to the prognosis of gastric carcinoma [27], breast cancer [30], cholangiocarcinoma [31] and urothelial carcinoma [29]. However, there are few studies on TANs in glioblastoma, especially for patients with glioblastoma, IDH wild-type, of which the prognostic value is currently unclear. What's more, at present, studies on hematologic markers of glioma mostly center on preoperative peripheral blood samples [32], and are often disturbed by many factors such as preoperative stress and postoperative infection, which can greatly limit the representation of the real postoperative condition of glioma. Correlative research on the influence of peripheral blood neutrophils on the overall survival of patients with glioma before postoperative radiotherapy has been reported less now, and its influence on the prognosis of glioma is of certain research value.

In the present study, RNA-sequencing (RNA-seq) expression profiles and clinical data from the TCGA database were used to measure the abundance of TANs in the tumor microenvironment by the CIBERSORTx algorithm, and to evaluate the relationship between TANs and clinical prognosis. Also, the CGGA database was used for validation. Moreover, the correlation between the functional molecules

involved in the tumor-promoting mechanism of neutrophils and the TANs was studied. In addition, a retrospective analysis was made on the levels of peripheral blood neutrophils before radiotherapy for patients with glioblastoma, IDH wild-type, and the prognostic significance of this metric was determined for glioblastoma, IDH wild-type.

Methods

1. Data collection

(1) TCGA database

Level 3 gene expression profiles (level 3 data) for glioblastoma patients were downloaded from TCGA (The Cancer Genome Atlas) database (https://portal.gdc.cancer.gov/). Clinical data such as sex, age, outcome, and survival time were also downloaded from TCGA data portal. The detailed inclusion criteria included: 1) primary glioblastoma; 2) WHO IV [33]; 3) IDH wild-type [33]. Exclusion criteria included: 1) recurrent glioblastoma; 2) incomplete records in grade or IDH mutation status; 3) patients with missing survival data or OS <90 days, or without definitive OS and outcome.

(2) CGGA database

The CGGA RNA sequencing (RNA-seq) dataset (mRNAseq_693, mRNAseq_325) and corresponding molecular and clinical information were acquired from the Chinese glioma genome atlas (CGGA) database (http://www.cgga.org.cn/index.jsp), which provides information such as age, sex, grade, subtype, MGMT promoter status, IDH status, and follow-up data of each patient. Inclusion and exclusion criteria were consistent with those for the TCGA dataset.

2. Acquisition of tumor-associated neutrophil data

By using TCGA and CGGA RNA-seq data, the content of glioblastoma, IDH wild-type TANs was computed by CIBERSORTx, an analysis tool (https://cibersortx.stanford.edu/) [34]. The content of TANs was considered a continuous variable, and a binary variable was obtained by establishing a cut-off point (cut) by using "survMisc" package, where TANs content below or equal to the cut-off point was considered as the low group, and the high group was patients whose TANs content was higher than the cut-off point.

3. Levels of functional molecules of tumor-promoting mechanisms of neutrophils

Studies have reported that many functional molecules, including ARG1, EGF, HGF, MMP9, PDGFB, S100A8, and S100A9, are involved in the tumor-promoting mechanism of neutrophils in the tumor micro-environment. Further analysis was made on the correlation between these seven functional molecules and TANs, as well as the disparity of functional molecules of tumor-promoting mechanisms of neutrophils among different TAN groups.

4. Evaluation of the peripheral blood neutrophils cohort of the cancer center

(1) Ethical approval of the study protocol

The study protocol was approved by the Ethics Review Board for Human Research of The First Affiliated Hospital of Fujian Medical University (Fujian, China), (approval No. [2015]084-1), and all participants gave written informed consent.

(2) Research design

A retrospective cohort study was adopted to collect data from all patients with glioblastoma, IDH wild-type treated in the radiotherapy department from September 2013 to June 2020. Inclusion criteria: 1) all patients were histopathologically diagnosed with glioblastoma, IDH wild-type; 2) surgery and post-operative intensity modulated radiation therapy (IMRT) were performed; 3) the hematological examination data was completed within one week prior to radiotherapy; 4) patients with complete follow-up information. Exclusion criteria: 1) antitumor therapy was performed before surgery (including radiotherapy, chemotherapy, biotherapy, immunotherapy, or targeted therapy); 2) patient suffered from an infectious diseases such as septicemia during hematological examination; 3) the presence of two or more tumors simultaneously; 4) complications with hematological diseases; 5) complications with immune system diseases; 6) transfusion history within 1 month; 7) history of long-term hormone therapy.

(3) Demographic and clinicopathologic variables and outcomes

Demographic and clinicopathologic variables included sex, age and methylation status of the MGMT promoter. The level of neutrophil counts in routine blood parameters within one week prior to radiotherapy were also reported. A cut-off point was obtained using the "survMisc" package, and the level of neutrophil counts were divided into a high group and a low group. Follow-up, including further consultation and / or telephone follow-up, ended in December 2020.

5. Statistical Analysis

R version 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria;www.r-project.org) was used for statistical analysis. The categorical variables were presented as number and percentage (N, %), and Pearson's Chi-Square test was used for comparison between groups. The correlation between the content of functional molecules and TANs, as mentioned above, was confirmed by Spearman correlation analysis. The overall survival (OS) was estimated from the date of diagnosis to death or the last follow-up, which was calculated by Kaplan-Meier method and the log-rank test. The univariate and multivariate Cox regression models were performed to determine potential prognostic factors. Sensitivity analysis: as to the TANs computed by CIBERSORTx, the results of multivariate Cox regression analysis of TANs infiltration were repeatedly validated, to verify the robustness of the determination of independent risk factors for high and low, identified by different in silico algorithms. Adopt E-value [35] was used to evaluate the extent to which confounding factors influenced the results. All statistical tests were two-sided, and a p-value of $P < 0.05$ was considered significant.

Results

1. Demographic and clinicopathologic characteristics

The study design is shown in **Figure 1**. A total of 113 eligible glioblastoma, IDH wild-type patients were identified in the TCGA database and selected in this study. In the TCGA dataset, there was no statistically significant difference in age, sex, whether radiotherapy or chemotherapy was performed, and MGMT promoter methylation status among patients in the TANs high group and low group ($P > 0.05$). Similarly, the CGGA RNA-seq database with 173 glioblastoma, IDH wild-type samples was used as a validation cohort. In CCGA dataset, there was statistically significant difference in chemotherapy among patients in the TANs high group and low group ($P < 0.05$). There were no statistically significant differences in age, sex, whether radiotherapy was performed, and MGMT promoter methylation status ($P > 0.05$)(**Table 1**).

2. Survival of patients and potential prognostic factors for OS

(1) TCGA dataset

In the TCGA dataset, clinical follow-up was available for 113 patients. The median survival time of patients in the TAN high group was 11.4 months, and was 15.1 months for patients in the TAN low group, and there was a statistically significant difference in overall survival between the groups ($\chi^2 = 6.0$, $P = 0.014$; **Figure 2A**). Of note, age, sex and MGMT promoter methylation status did not significantly affect OS ($P= 0.972$, 0.987 and 0.271; **Figure 2B-D**). Meanwhile, patients received radiotherapy or chemotherapy may had longer OS ($P= 0.037$ and $P< 0.001$; **Figure 2E, F**). In the TCGA dataset, univariate Cox analysis have shown that the infiltration of TANs (HR = 1.931, 95% CI: 1.139-3.274), radiotherapy (HR = 0.339, 95% CI: 0.187-0.616), and chemotherapy (HR = 0.610, 95% CI: 0.381-0.977) were factors that significantly influenced the prognosis of patients with glioblastoma, IDH wild-type **(Figure 2G)**. Multivariate Cox regression showed that the infiltration of TANs (HR = 1.811, 95% CI: 1.048-3.128) and radiotherapy (HR = 0.381, 95% CI: 0.163-0.889) were independent factors influencing the prognosis of patients with glioblastoma, IDH wild-type.

(2) External Validation

In the CGGA dataset, follow-up details were available for 173 patients. The median survival time of patients in the TAN high group was 12.6 months, and was 15.8 months for patients in the TAN low group; there were statistically significant differences in overall survival between the two groups ($\chi^2 = 9.3$, $P = 0.002$; **Figure S1A**). In the CGGA dataset, univariate Cox analysis revealed that the infiltration of TANs (HR = 1.799, 95% CI: 1.227-2.637) and chemotherapy (HR = 0. 419, 95% CI: 0.285-0.616) were factors influencing the prognosis of patients with glioblastoma, IDH wild-type (**Figure S1G**). Multivariate Cox regression showed that the level of tumor-associated neutrophil infiltration (HR = 1.572, 95% CI: 1.046-2.363) and chemotherapy (HR = 0.406, 95% CI: 0.264-0.625) were independent prognostic factors for OS of glioblastoma and IDH wild-type patients.

3. Stratification Analysis

In the TCGA dataset, stratification analysis was performed based on age, sex, chemotherapy, and MGMT promoter methylation status. Among 113 patients aged ≤ 60 years, the median survival time of patients in the TAN high group was 12.0 months, and was 16.9 months for patients in the TAN low group, and a statistically significant difference in overall survival was identified between the two groups (χ^2 = 14.6, P 0.001). In females, the median survival time of patients in the TAN high group was 5.83 months, and was 15.33 months for patients in the TAN low group, and there was a statistically significant difference in overall survival between the two groups, stratified by patient sex (χ^2 = 8.9, P = 0.003). Among patients who received no chemotherapy, the median survival time of patients in TAN high group was 4.73 months, and that of patients in the TAN low group was 10.97 months, indicating a statistically significant difference in overall survival between the two groups stratified by chemotherapy status (χ^2 = 5, P = 0.03). In patients with MGMT promoter methylation, the median survival time of patients in the TAN high group was 11.4 months, and was 16.3 months for patients in the TAN low group, indicating a statistically significant difference in overall survival between the two groups stratified by MGMT promoter methylation status (χ^2 = 9.5, P = 0.002). According to the univariate analysis, there was an interaction between neutrophil infiltration content, patient sex, and MGMT promoter methylation status (**Figure 3**).

In the CGGA dataset, stratification analysis was performed based on patient age, sex, radiotherapy status, and MGMT promoter methylation status. Among 173 patients aged ≥ 56 years, the median survival time of patients in the TAN high group was 9.0 months, and was 13.3 months for patients in the TAN low group, indicating a statistically significant difference in overall survival between the two groups (χ^2 = 10.7, P = 0.001). In male patients, the median overall survival time of in the TAN high group was 12.7 months, and was 15.8 months in the TAN low group (χ^2 = 8.1, P = 0.004). For patients receiving radiation therapy, the median survival time in the TAN high group was 13.1 months, and was 15.8 months for patients in the TAN low group, indicating a statistically significant difference in overall survival between the two groups subset by radiation therapy status (χ^2 = 6.3, P = 0.01). Among patients with MGMT promoter methylation, the median survival time was 12.6 months in the TAN high group and 18.2 months in the TAN low group, indicating a statistically significant difference in overall survival between the two groups subset by MGMT promoter methylation status (χ^2 = 4.4, P = 0.04). Based on the univariate analysis, there was an interaction between neutrophil infiltration, radiotherapy, and MGMT promoter methylation status **(Figure S2**).

4. Sensitivity coefficient analysis

In the TCGA dataset, after adjusting for patient age, sex, radiation, chemotherapy, and methylation of MGMT promoter, the RR = 1.507 and E-value = 2.38 were determined for death in the TAN high (**Figure 4**). In the CGGA dataset, the RR = 1.572 and E-value = 2.52 were determined for death in the TAN high group (**Figure S3**).

5. Assessment of functional molecules of tumor-promoting mechanisms of neutrophils and infiltration of TANs

The linear correlation between levels of ARG1, EGF, HGF, MMP9, PDGFB, S100A8, and S100A9 and the metric of infiltration of TANs was determined by correlation coefficients. Correlation coefficient analysis demonstrated that ARG1, PDGF, MMP9, S100A8, and S100A9 were linearly correlated with infiltration of TANs (**Figure 5A-G**). Compared to the TAN low group, the expression of ARG1, S100A8, and S100A9 was significantly increased in the TAN high group (**Figure 5H-N**).

6. Patient characteristics of the peripheral blood neutrophils cohort

In the peripheral blood neutrophils cohort, 143 patients with glioblastoma, IDH wild-type were included, and there were no statistically significant differences in age, sex, radiation, chemotherapy, or methylation of MGMT promoter between the peripheral blood neutrophil high and low groups before radiation **(Table S1)**.

The correlation between peripheral blood neutrophils and survival before radiation was analyzed; 50 patients died at the end of follow-up, with a median survival time of 21.8 months in the peripheral blood neutrophil high group, and 13 patients died in the low group, with a median survival time of 39.4 months. The overall survival of patients in the high group was significantly shorter than that in low group ($\chi^2 = 4.9$, $P = 0.026$; **Figure 6A**). **Figure 6B-D** presents the KM curves for patients with different age, sex and MGMT promoter methylation status. In accordance with the univariate and multivariate Cox regression models: the level of peripheral blood neutrophils before radiation (HR = 2.073, 95% CI: 1.077-3.990) was an independent risk factor affecting the overall survival of patients with glioblastoma, IDH wild-type (**Figure 6E**).

Discussion

To date, most studies on the clinical prognostic value of neutrophils in glioblastoma are centered on the prognostic value of NLR, but there is limited data about the prognostic relevance of TANs in glioblastoma, especially for patients with glioblastoma, IDH wild-type. Based on the RNA-seq data from the TCGA and CGGA datasets, the CIBERSORTx algorithm was used to infer TAN infiltration levels. We report that the level of infiltration of TANs is an independent prognostic factor for survival in patients with glioblastoma, IDH wild-type, and that higher levels of TANs associates with poorer overall survival, which is consistent with other reports [32, 36]. Compared to patients with low infiltration of TANs, the expression of neutrophil tumor-promoting molecules ARG1, S100A8, and S100A9 was significantly increased in patients with in high infiltration of TANs. Additionally, data from 143 patients with glioblastoma, IDH wild-type were analyzed in this study, to investigate the prognostic value of peripheral blood neutrophils before radiotherapy on overall survival of patients with glioblastoma, IDH wild-type. We found that the level of peripheral blood neutrophils prior to radiotherapy was an independent prognostic factor of overall survival for patients with glioblastoma, IDH wild-type. According to stratification analysis in the TCGA dataset, female patients aged ≤ 60 years old with high TAN levels, who had not previously received chemotherapy, had a worse prognosis. In the CGGA dataset, male patients aged ≥ 56 years old with high TAN levels, with an unmethylated and/or unknown methylation status of the MGMT promoter, had a

worse prognosis. There were also statistically significant differences in the overall survival of patients in the TAN high group and TAN low group, with or without radiotherapy.

Most current studies on the prognostic significance of TANs do not agree on the relevant biomarkers of neutrophils, which may result in a bias in prognostic estimates. By analyzing three groups of operative specimens of patients with gastric cancer who received total or partial gastrectomy independently at two medical centers, Zhang *et al.* found that high infiltration of TANs in gastric tissue suggests a better prognosis [27]. Zhao *et al.* [37] demonstrated that high infiltration of TANs in gastric tissue suggests a poor prognosis. Causes for this difference may be that CD66b was used to mark neutrophils in the former study, while CD15 was used to mark neutrophils in the latter study. CD15 can be expressed not only in neutrophils, but also in monocytes, eosinophils, and tumor cells, among other cell types. As a consequence, the RNA-seq data of TCGA and CGGA datasets were analyzed in the present study by CIBERSORTx in an exploratory way, to infer the neutrophil infiltration levels and avoid potential biases introduced by evaluating only specific neutrophil markers.

TANs are involved in malignant transformation and angiogenesis in numerous preclinical and clinical studies [36, 38–41]. By releasing angiogenic factors including BV8 and S100 proteins S100A8 and S100A9, as well as activating vascular endothelial growth factors A (VEGFA) in the extracellular matrix and MMP9, tumor angiogenesis was maintained by neutrophils [11–14]. This angiogenic effect was also found in hepatocellular carcinoma, gastric cancer, and nasal carcinoma [42–44]. Transcriptional analysis of neutrophils in spleen and blood of mice with breast cancer and without tumors, showed that the transcriptional process of neutrophils in mice with tumors was altered, and that molecules such as G-CSF (granulocyte colony-stimulating factor) and TGF (transforming growth factor) β in the tumor microenvironment induce neutrophils to express arginase 1 (Arg1), reactive oxygen species (ROS), and nitric oxide (NO), and shifted neutrophils to an immunosuppressive phenotype [15, 45, 46] that inhibited T-cell activation [15–17]. The tumor-promoting activity of neutrophils was related to growth factors and chemotactic factors [36, 47, 48], and CXCR was involved in promoting neutrophil maturation, survival, and recruitment [15, 49–51]. Neutrophils promoted the survival and exudation of circulating tumor cells through integrin-mediated direct interactions [52, 53]. Granulocyte-macrophage colony stimulating factor (GM-CSF) was found to induce the expression of fatty acid transport protein 2 (FATP2) in neutrophils. While FATP2 may promote the uptake of arachidonic acid and synthesis of prostaglandin E2(PGE2) [18], the use of a FATP2 inhibitor in tumor-bearing mice may reduce the immunosuppressive activity of neutrophils and enhance tumor growth [18]. By producing epidermal growth factor (EGF), hepatocyte growth factor (HGF), and platelet-derived growth factor (PDGF) [25, 26], neutrophils may release neutrophil extracellular traps (NET), neutrophil elastase (NE), and matrix metalloproteinase-9 (MMP-9), as well as cleaved laminin-111 [23, 24], to stimulate dormant cancer cells [24] and to produce epitopes that bind to tumor integrins, thus triggering the proliferation of cancer cells [24]. Furthermore, by promoting the transformation of endothelial cells into mesenchymal cells, NET can [54, 55] promote angiogenesis [13, 56–58], cancer-related thrombogenesis [59], and prevent mechanisms such as CD8 + and natural killer (NK) cell-mediated cytotoxicity [60] from being involved in promoting tumor growth.

Neutrophils are classical congenital immune cells that are important members of the tumor immune micro-environment. Neutrophils in peripheral blood and tissues are of the same origin [61, 62], and the correlation between neutrophils and tumors prognosis remains controversial [63–70]. Most clinical studies are primarily concerned with preoperative NLR [28, 29, 32, 71, 72], which may not truly reflect the prognostic value of peripheral blood neutrophils, given that this index is susceptible to lymphocyte interference. K. Takakura *et al*. [73] demonstrated that NLR was significantly associated with high density CD20 + lymphocytes (P = 0.031) and CD163 + macrophagocytes (P = 0.023), but not with CD66b + neutrophils (P = 0.397). Also, the correlation between neutrophils and prognosis may also be influenced by the location in tumors. Immunohistochemical studies on operative specimens of esophageal squamous carcinoma found that 5-year rates of DFS and OS were 20% and 26.7%, respectively, in patients with increased CD66 + intratumoral neutrophils, but 51.1% and 55.5%, respectively, in patients with decreased CD66 + neutrophils, suggesting that CD66 + neutrophils are an independent prognostic factor of DFS [HR = 2.174 (1.249–3.784), P = 0.006] and OS [HR = 1.858 (1.038–3.325), p = 0.037]. No prognostic significance of peritumoral neutrophils was noted [70]. The correlation between neutrophils and prognosis was also influenced by the time of specimen collection, especially peripheral blood specimens. Whereas most patients with glioma are treated with surgery, there may be differences in tumor burden status after operation compared with pre-operation. Therefore, the time point before radiotherapy used in this study, with strict inclusion and exclusion criteria to avoid the influences brought by postoperative surgical stress or postoperative infection, may ensure better evaluation of the effects of the overall immune status of patients with glioma before radiotherapy. Our results showed that the level of peripheral blood neutrophils before radiotherapy was an independent risk factor that affects the prognosis of patients with glioblastoma, IDH wild-type, suggesting that immune status before radiotherapy affects the survival of patients with glioma.

Although this study performed an exploration on the correlation between neutrophil infiltration and prognosis of patients with glioblastoma, IDH wild-type, based on TCGA and CGGA, and also explored the prognostic value of pre-treatment neutrophil levels in these patients, there are still many limitations: 1) this was a retrospective study, and TAN were informed from CIBERSORTx by evaluation of RNA-seq data; 2) there lacked information regarding TAN levels from the center for validation; 3) the study did not evaluate in-depth the interactions between neutrophils in blood and in pathological tissues, and tumor promotion, or mechanisms of inhibition; 4) complete chemotherapy records were not available for the entire cohort. The results of this study need to be validated by prospective multi-center randomized trials with a larger patient population in the future.

Conclusions

TANs can be used as a prognostic marker for patients with glioblastoma, IDH wild-type. Patients whose tumors have a high infiltration of TANs have a worse prognosis.

Declarations

Supplementary Information

The online version contains supplementary material available.

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Authors' contribution

Xuezhen Wang: Writing – original draft (lead); writing – review and editing (equal input); formal analysis (equal input).

Xiaoxia Li: Visualization (equal input); formal analysis (equal input).

Yufan Wu: Visualization (equal input); formal analysis (equal input).

Jinsheng Hong: Writing – review and editing (equal input).

Mingwei Zhang: Conceptualization (lead); writing – review and editing (equal input).

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Ethical Review Board for Human Research of The First Affiliated Hospital of Fujian Medical University, Fujian, China (approval No. [2015]084-1), and all participants gave written informed consent. Part of data in this study were downloaded from the TCGA and CGGA databases hence it was no additional ethics approval was necessary. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

All authors of this paper have read and approved the final version submitted.

Data availability statement

Data are available from the corresponding authors on reasonable request.

Competing of interests

The authors declare that they have no competing interests

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Tables

Table 1. Summary of clinicopathological features of glioblastoma, IDH wild-type patients in the TCGA and CGGA cohorts.

	TCGA			CGGA		
Variables	Total (n=113)	High group (n=22)	Low group (n=91)	Total (n=173)	High group (n=38)	Low group (n=135)
Age, median	62	62	62	55	57.5	55
Sex						
Female	38 (34%)	7 (32%)	31 (34%)	68 (39%)	13 (34%)	55 (41%)
Male	75 (66%)	15 (68%)	60 (66%)	105 (61%)	25 (66%)	80 (59%)
Radiation						
NO	13 (12%)	5 (23%)	8 (9%)	25 (14%)	3 (8%)	22 (16%)
YES	100 (88%)	17 (77%)	83 (91%)	148 (86%)	35 (92%)	113 (84%)
Chemotherapy						
NO	27 (24%)	5 (23%)	22 (24%)	37 (21%)	14 (37%)	23 (17%)
YES	86 (76%)	17 (77%)	69 (76%)	136 (79%)	24 (63%)	112 (83%)
MGMT promoter						
Methylated	37 (33%)	7 (32%)	30 (33%)	71 (41%)	12 (32%)	59 (44%)
Un-methylated/ Unknown	76 (67%)	15 (68%)	61 (67%)	102 (59%)	26 (68%)	76 (56%)

Figures

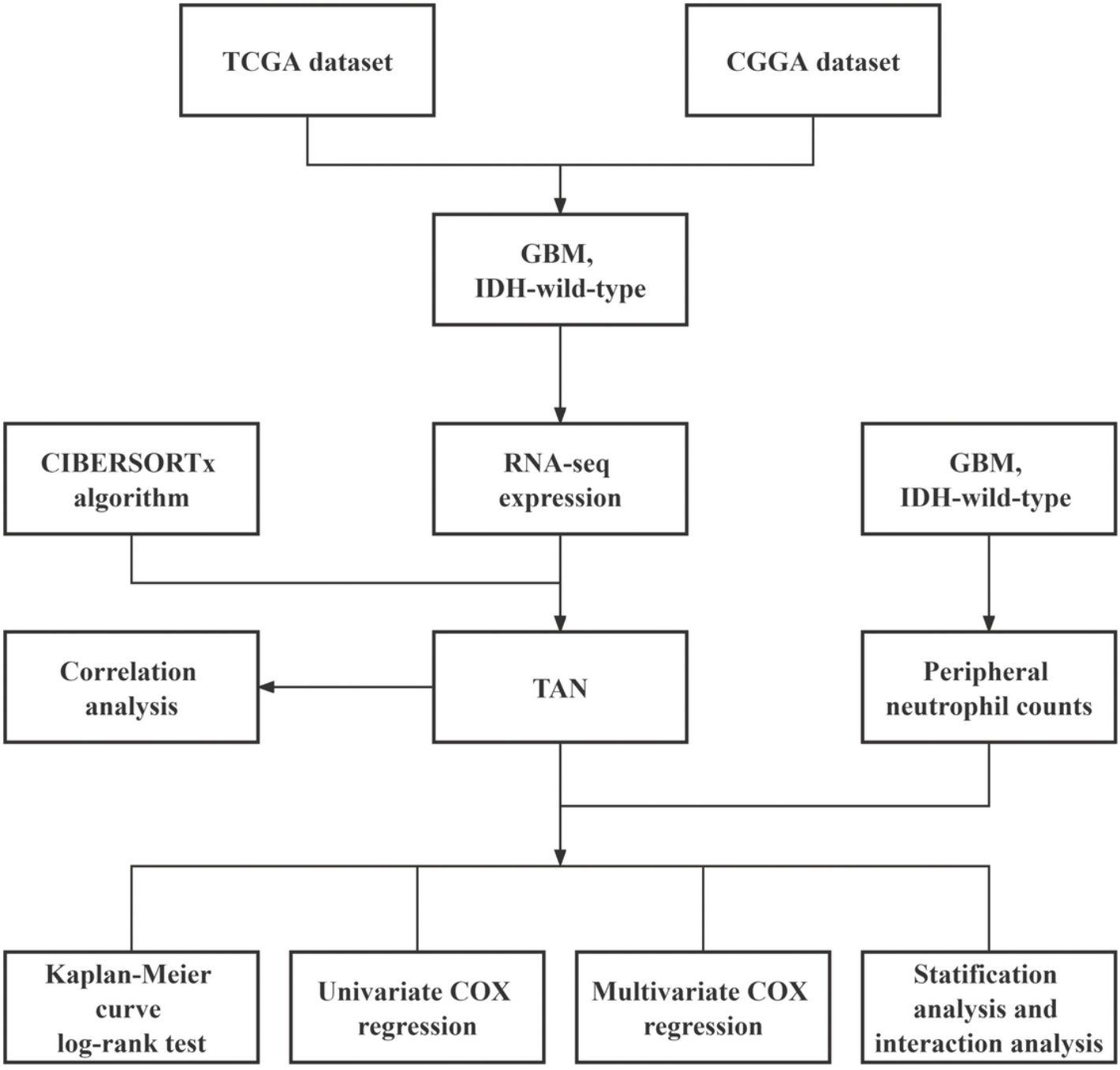

Figure 1

Flowchart of sample data analysis.

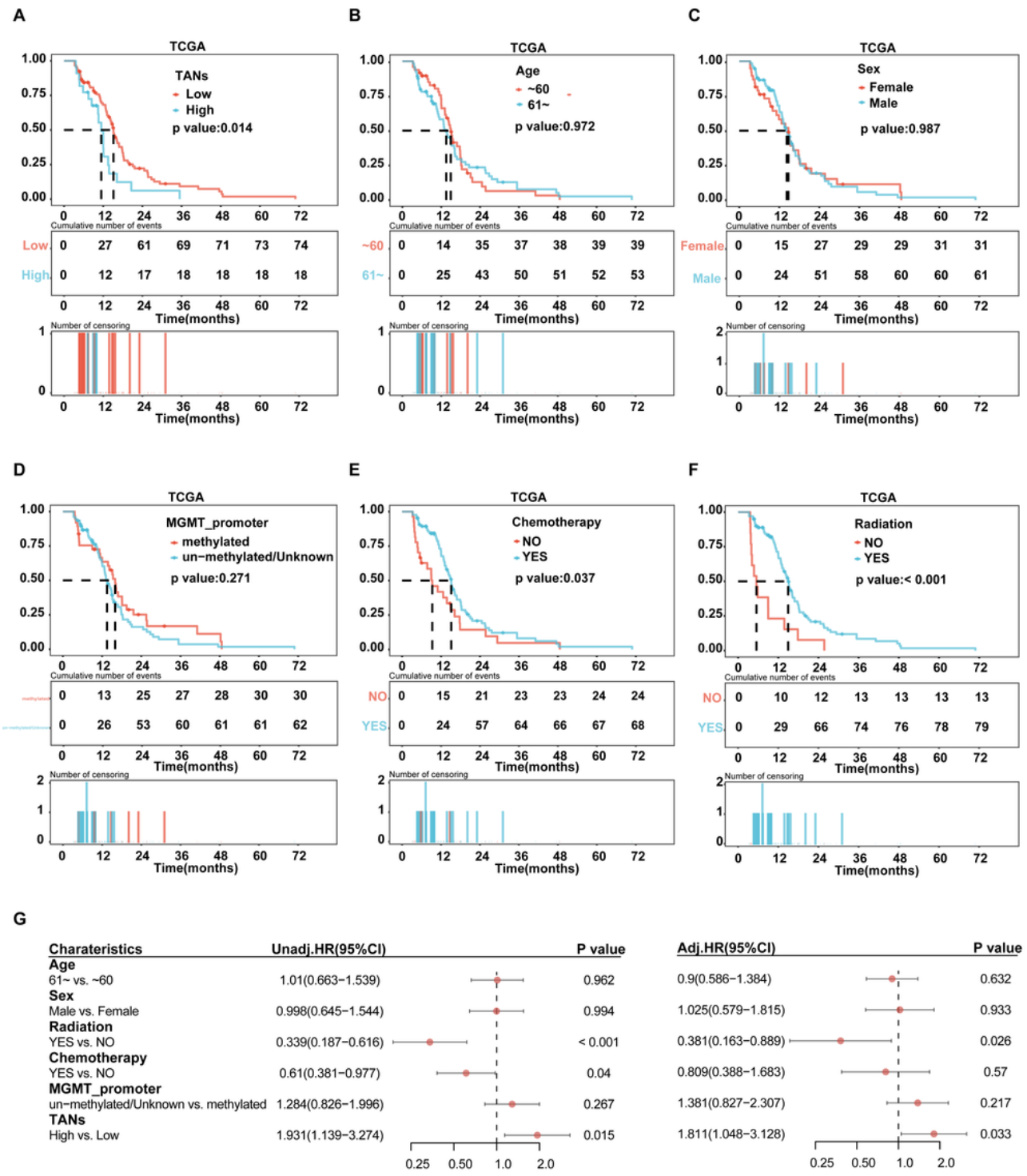

Figure 2

KM survival curves of patients based on TAN levels **(A)**, age **(B)**, sex **(C)**, MGMT-promoter status **(D)**, chemotherapy status **(E)**, radiation status **(F)**, and univariate and multivariate Cox analyses **(G)** of TAN levels and patient survival in the TCGA cohort.

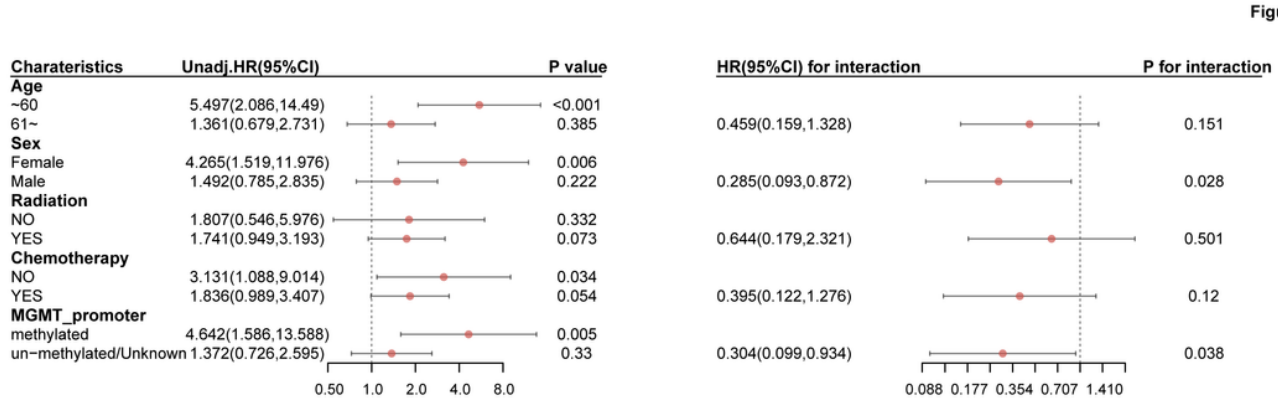

Figure 3

Subgroup analyses and subgroup interactions in the TCGA cohort.

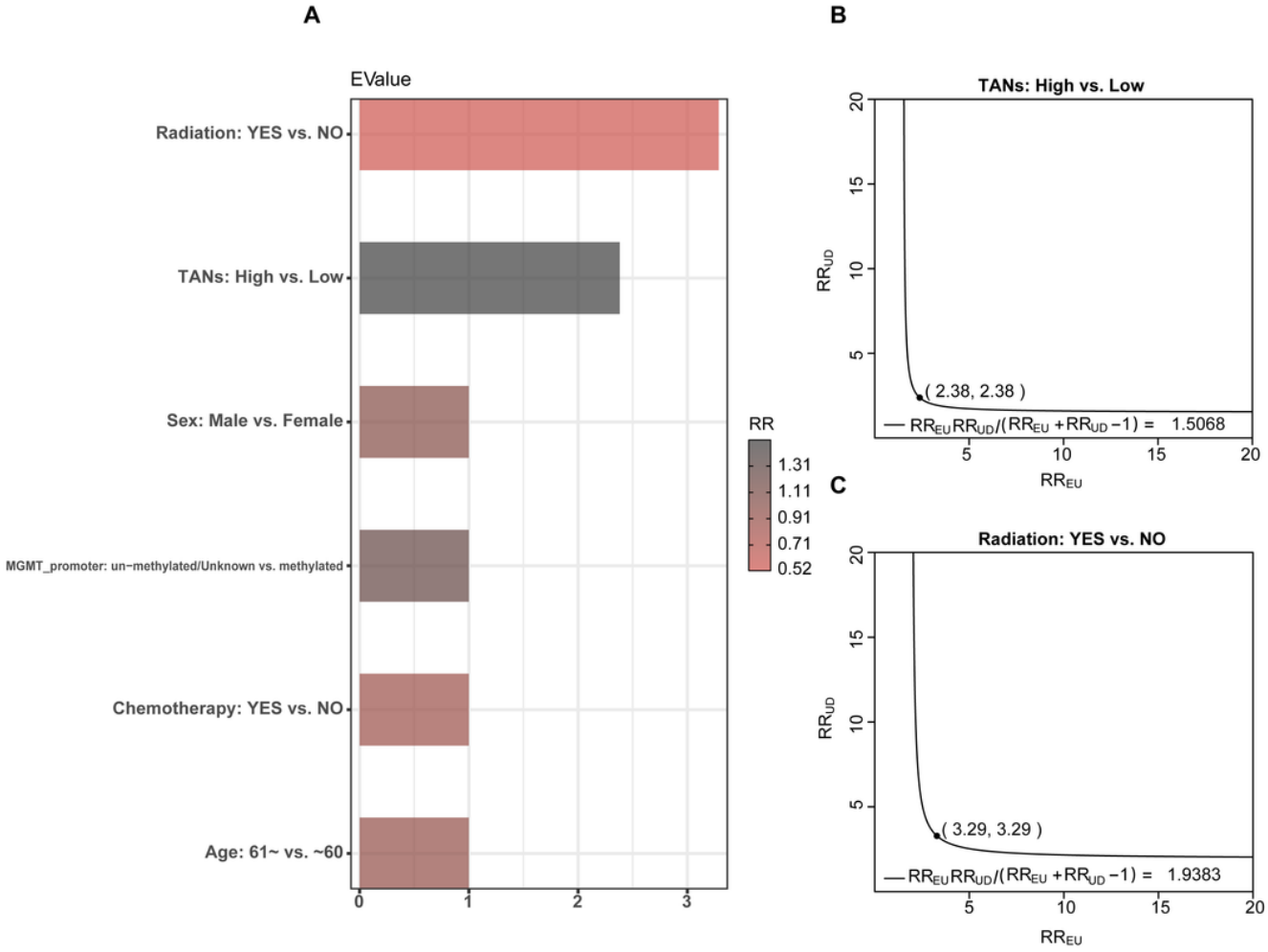

Figure 4

Sensitivity analyses in the TCGA cohort.

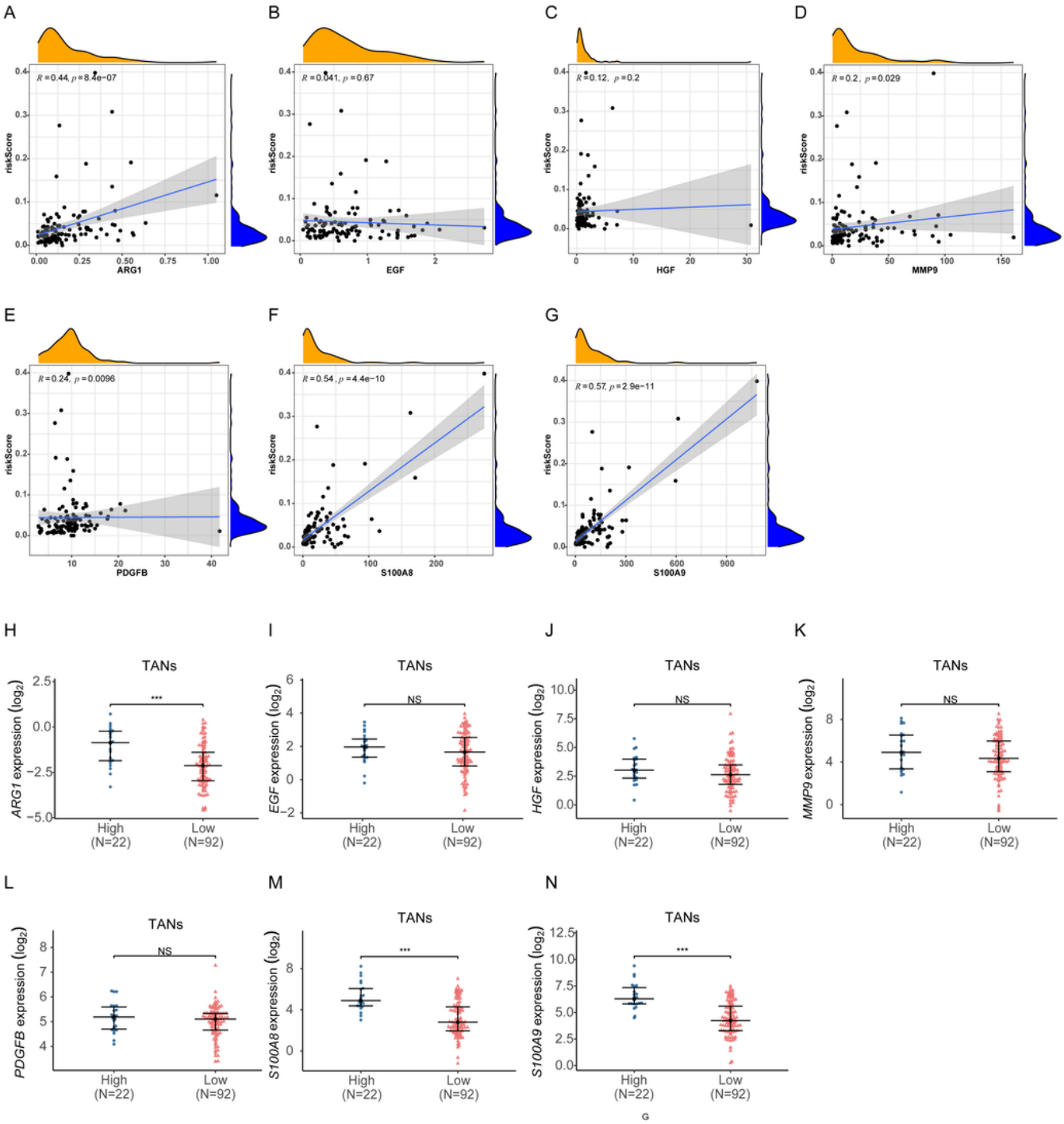

Figure 5

Correlation analysis between TAN and ARG1 **(A)**, EGF **(B)**, HGF **(C)**, MMP9 **(D)**, PDGFB **(E)**, S100A8 **(F)**, and S100A9 **(G)**. Expression of ARG1**(H)**, EGF **(I)**, HGF **(J)**, MMP9 **(K)**, PDGFB **(L)**, S100A8 **(M)**, and S100A9 **(N)** in the TAN high and low groups.

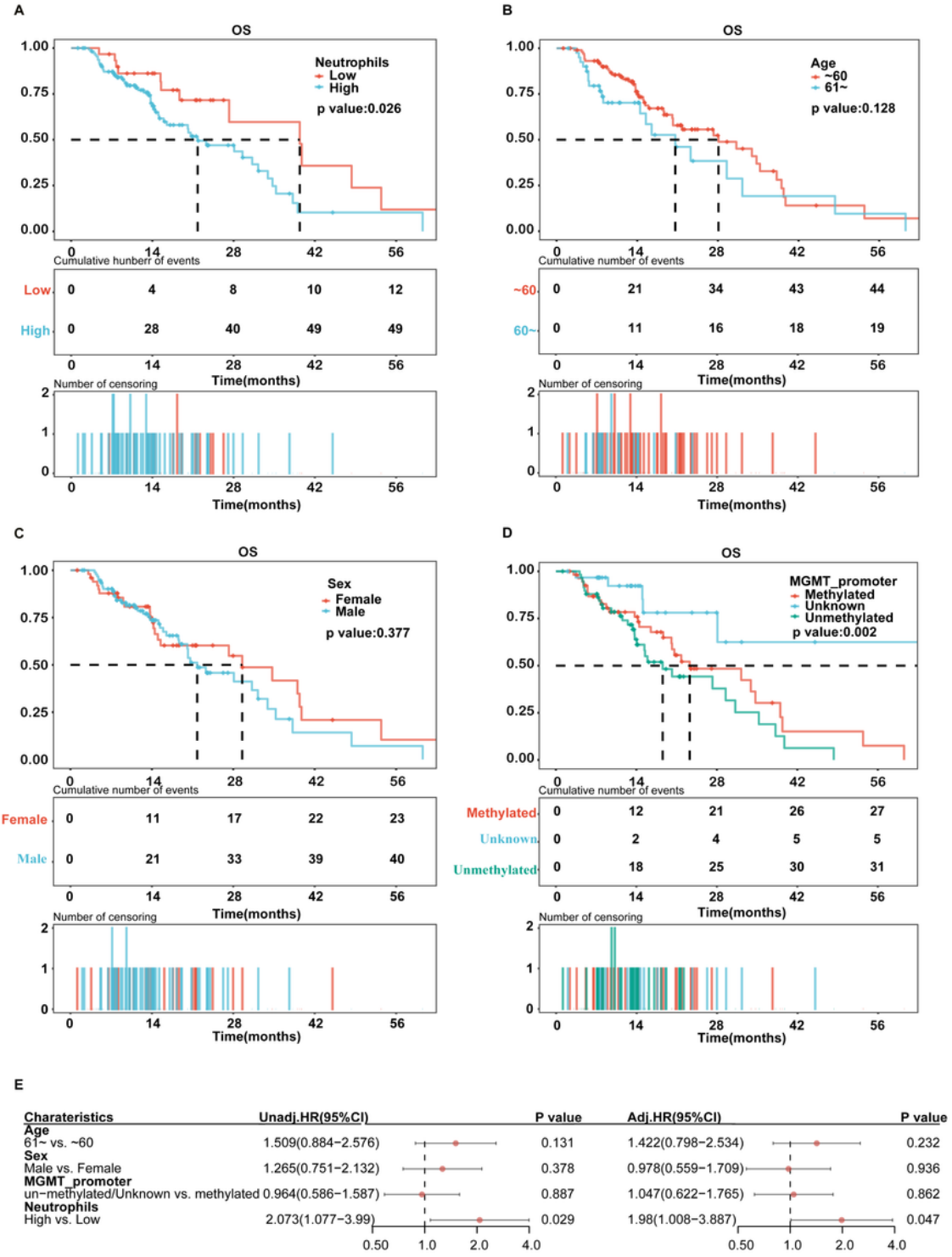

Figure 6

KM survival curves of peripheral blood neutrophils **(A)**, age **(B)**, sex **(C)**, MGMT-promoter status **(D)**, and univariate and multivariate Cox analyses **(E)** of peripheral blood neutrophils before radiation.

Supplementary Files

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