Identification of a brand intratumor microbiome signature for predicting prognosis of hepatocellular carcinoma

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Abstract

Purpose

Given that prognosis of hepatocellular carcinoma (HCC) differs dramatically, it is imperative to uncover effective and available prognostic biomarker(s). The intratumor microbiome plays a significant role in the response to tumor microenvironment, we aimed to identify an intratumor microbiome signature for predicting the prognosis of HCC patients accurately and investigate its possible mechanisms subsequently.

Methods

The TCGA HCC microbiome data (TCGA-LIHC-microbiome) was downloaded from cBioPortal. To create an intratumor microbiome related prognostic signature, univariate and multivariate Cox regression analyses were used to quantify the association of microbial abundance and patients' overall survival (OS), as well as their diseases specific survival (DSS). The performance of the scoring model was evaluated by the area under the ROC curve (AUC). Based on the microbiome related signature, clinical factors, and multi-omics molecular subtypes on the basis of "icluster" algorithm, nomograms were established to predict OS and DSS. Patients were further clustered into three subtypes based on their microbiome related characteristics by consensus clustering. Moreover, deconvolution algorithm, weighted correlation network analysis (WGCNA) and gene set variation analysis (GSVA) were used to investigate the potential mechanisms.

Results

In TCGA LIHC microbiome data, the abundances of 166 genera among the total 1406 genera were considerably associated with HCC patients' OS. From that filtered dataset we identified a 27-microbe prognostic signature and developed a microbiome related score (MRS) model. Compared with those in relatively low risk group, patients in higher risk group own a much worse OS($P < 0.0001$). Besides, the time-dependent ROC curves with MRS showed excellent predictive efficacy both in OS and DSS. Moreover, MRS is an independent prognostic factor for OS and DSS over clinical factors and multi-omics based molecular subtypes. The integration of MRS into nomograms significantly improved the efficacy of prognosis prediction (1 year AUC:0.849, 3 year AUC: 0.825, 5-year AUC: 0.822). The analysis of microbiome-based subtypes on their immune characteristics and specific gene modules inferred that intratumor microbiome may affect the HCC patients' prognosis via modulating the cancer stemness and immune response.

Conclusion

MRS, a 27 intratumor microbiome related prognostic model, was successfully established to predict HCC patients overall survive independently. And the possible underlying mechanisms were also investigated to provide a potential intervention strategy.

Introduction

Great progress in the diagnosis and treatments of human hepatocellular carcinoma (HCC) has been made to ameliorate the patients' suffering, however, HCC still occurs to be the second and fifth estimated leading cause of death in China and US(Q. Li et al., 2022; Xia et al., 2022). Due to the complexity and heterogeneity of HCC,

variate responses to the treatments lead to significant disparate burden among the patients(Luo et al., 2021). Thus, it is imperative to identify new therapeutic and prognostic biomarkers, as well as the more powerful targets for treatments. Basically, the current biomarkers were mainly established on cellular related profiles, such as genomic and proteomic profiles. Microbiome, one population of special commensal, has arisen remarkable interest over therapeutic strategies in cancer, especially for personal medical therapies. Despite increasing evidence on the importance of gut microbiome in cancer(Helmink et al., 2019) and other gastrointestinal diseases(Hajj Hussein et al., 2023), clinical effects of intratumor microbiome with its mechanism have not been fully explored.

There are an increasing number of researches aimed at grasping the significance of the microbiome in HCC development and progression. Gut microbiome and oral microbiome have been identified to be a non-invasive diagnostic biomarkers for HCC(Rao et al., 2020). Using mouse models, leaky gut and dysbiosis in the gut microbiota have been interacted with HCC since the subpopulation of gut bacteria could alter the production of certain metabolites or microbiota-associated molecular patterns, and augment the systematic LPS level at different stages of HCC development in mice, which leads to the chaos of immune microenvironment(Dapito et al., 2012; Gäbele et al., 2011; Ma et al., 2018). Interestingly, recent investigations also revealed that both the gut and tissue-resident microbiota are able to promote tumor metastasis(Fu et al., 2022; R. Li et al., 2019). Moreover, distinct gut microbiome characteristics has been found in HCC tissue in comparison to healthy breast tissue, which may regarded as non-invasive biomarkers for HCC diagnosis and potential targets for HCC prevention(Kang et al., 2022; Ren et al., 2019). Researchers referred that Bacteroides, Lachnospiracea incertae sedis and Clostridium XIVa appear to be enriched in HBV-related HCC patients with a high tumor burden(H. Huang et al., 2020), indicating that these microbes may exert negative effects in these patients. All these findings offer evidence of a significant relationship between the microbiome and the carcinogenesis and progression of HCC.

In addition, investigations also show that the microbiome plays a significant role in the patients' sensitivity to radiotherapy, chemotherapy and immunotherapy for cancer(Al-Qadami et al., 2019; Behary et al., 2021; Chiba et al., 2020). Given that immunotherapy is part of the first line treatment for HCC, it is worth noting that microbiome has a dual role in tumor immune response. For instances, with germ-free or antibiotic treated mice, researchers found that fecal transplantation from patients who respond to immune checkpoint blockade could improve the antitumor effect of PD-1 inhibitor(Routy et al., 2018). On the other side, immunostimulatory role of Bifidobacterium and B fragilis was further proved, which augment the efficacy of anti–PD-L1 and anti–CTLA-4 immunotherapy(Sivan et al., 2015; Vétizou et al., 2015). In total, these results imply that the microbiome is prospective for both diagnosis biomarkers and treatment targets.

The purpose of this study was to identify an intratumor microbiome related signature for establishing a prognostic scoring system, assessing its clinical impact using TCGA LIHC microbiome data, and attempt to investigate the mechanism beneath this tumor commensal.

Methods and materials

Collection of PAC datasets and preprocessing

The Cancer Genome Atlas (TCGA) liver hepatocellular carcinoma microbiome (TCGA-LIHC-microbiome) was downloaded from the cBioPortal (https://www.cbioportal.org/)(Cerami et al., 2012). mRNA, miRNA, copy number variation and DNA methylation statistics of TCGA HCC patients with their clinical information were downloaded from UCSC Xena (http://xena.ucsc.edu/). Specially, microbial profile was established from whole-transcriptome sequencing filtering and analyzing of 356 HCC samples(Poore et al., 2020), where total of 1406 genera were detected and quantified in HCC. In addition, “iClusterPlus” (version 1.26.0) package was used to analyze the molecular subtypes of HCC patients by integrating multi-type genomic data(Mo et al., 2018).

Microbial signature and prognostic scoring model

For the microbiome related scoring (MRS) model construction, a systematic analysis was conducted step by step in the TCGA-LIHC-microbiome dataset: (1) Univariate Cox regression analysis (survival package in R, Version 3.4-0) was used to identify so- called OS related microbes from the 1406 candidates whose abundance were remarkably related to patients’ OS. OS-related microbes were further analyzed via Kaplan-Meier analysis (survminer package in R, Version 0.4.9) and log-rank test (survival package in R, Version 3.4-0), where TCGA-LIHC cohort was divided into high and low abundance groups for each microbe (survminer package in R, Version 0.4.9). Subsequently, (2) A forward conditional multivariate Cox regression (survival package in R, Version 3.4-0) for analysis of OS-related microbes was performed to select a set of independent microbes, regarded as the microbiome related signature. In this study, we identified a 27-microbe prognostic signature, then (3) we constructed the MRS model by the following formula where the coefficients were obtained from Cox regression analysis of the microbiome prognostic signature:

$$\mathrm{MRS} = \sum_{i=1}^{27} (\mathrm{coefficientofmicrobei}) \times (\mathrm{abundanceofmicrobei})$$

All patients were divided into three subtypes via X-tile software which was applied to select best cut points. The multivariate Cox regression was used to assess the independent prognostic impact of MAPS by adjusting for the clinical factors (age, stage, AFP level) and icluster-based molecular subtype.

Nomogram

A nomogram model was generateed to assist the prediction of 1-, 3-, and 5-year OS and disease-specific survival (DSS) rate of HCC patents (rms package in R, Version 6.4-1). The performance of the nomogram model was evaluated based on the time-dependent receiver-operating characteristic (ROC) curve (survivalROC package in R, Version 1.0.3.1).

Microbial cluster, WGCNA and functional enrichment analysis

using the “ConsensusClusterPlus” package (version 1.62.0), unsupervised clustering was applied to classify patients into 3 distinct clusters according to their intratumor microbiome abundance, repeated 1000 times to ensure classification stability(Wilkerson & Hayes, 2010). Moreover, to identify the key module correlated with the three clusters and construct module-trait relationships, Further, to identify co-expressed gene networks, the WGCNA R package (version 1.72-1) was employed to analyze the clustered microbiome-based subtypes(Langfelder & Horvath, 2008). The median absolute deviation (MAD) top 5000 genes were screened for network constructions with a soft thresholding power β = 6 and minModuleSize was set as 30.

Subsequently, parameters of hub genes of the specific module were set as gene significance (GS, Pearson's correlation between each gene and clinical trait) > 0.1 and module membership (MM, correlation between each gene and module) > 0.8. Afterward, functional enrichment analysis of module related hub genes were performed by clusterProfiler R package based on Kyoto Encyclopedia of Genes and Genomes (KEGG) (version 4.6.1)(Yu et al., 2012). The stemness of each tumor sample was quantified by ssGSEA, and the stemness signatures were collected as previously described(Zheng et al., 2022) [12].

Tumor microenvironment (TME) infiltrations exploration and immune response prediction

CIBERSORT, one of the robust deconvolution algorithms which quantify the relative proportions of 22 immune cells on the basis of normalized bulk sample's gene expression profiles(Newman et al., 2015). Additionally, ESTIMATE algorithm could assess immune and stromal cellular infiltrations in tumor samples(Yoshihara et al., 2013). Thus, we quantified the TME fractions of each HCC sample via "CIBERSORT" R script with 1,000 permutations and calculate the immune and stromal score through ESTIMATE in R package (version 1.0.13). Tumor Immune Dysfunction and Exclusion (TIDE), a reliable online algorithm (http://tide.dfci.harvard.edu/) was used to model tumor immune evasion as a higher TIDE score refers to a poorer immune response and worse immune evasion. Therefore, we employed it to estimate immunotherapeutic responses of each HCC patient(Jiang et al., 2018).

Statistical analysis

All statistical results were analyzed through R (version 4.2.2, https://www.r-project.org/). Normally distributed variables were analyzed by the student's t-test or Anova test. Non-normally distributed variables were analyzed via the Wilcoxon rank-sum test or Kruskal-Wallis test. Survival analysis was performed via the Kaplan-Meier method and the cox proportional hazards model to analyze associations between factors and prognosis by "survival" (version 3.4-0) and "survminer" (version 0.4.9) packages. Graphic visualizations were performed in R (ggplot2 package, version 3.4.1; ggpubr package, version 0.5.0). The difference was defined as statistically significant when adjusted $p < 0.05$ (two-tailed).

Results

Establishment of microbiome related model for hepatocellular carcinoma

By applying univariate Cox regression analysis, we identified 166 genera from the total 1046 genera detected in TCGA-LIHC dataset, among which 139 were favorable factors (Hazard ratio (HR) < 1, $P < 0.05$) and 27 were risk factors (HR > 1, $P < 0.05$) for patients' OS (Fig. 1A). Moreover, to evaluate the relationship between microbiome and OS, TCGA-LIHC patients were divided into two genera-abundance-based groups via the best cut point optimized by the most significant variance in OS. The specific effects of microbiome abundance on OS were estimated through Kaplan–Meier curves and log-rank test. To name only a few examples, the OS in patients whose tumor owns a higher abundance of Actinotignum and Dolosigranulum is remarkably longer (Fig. 1B-C) while in patients whose tumor owns a high abundance of Francisella and Actinobacillus is significantly shorter (Fig. 1D-E).

Further, 27 microbes were selected from TCGA-LIHC microbiome dataset using a forward stepwise multivariate Cox analysis. These genera were identified to exert significantly independent effect on OS. For prognosis risk microbes, higher abundance of Holophaga, Ornithinimicrobium, Sediminibacterium, Roseivirga, Pantoea, Candidatus Contendobacter, Caldimonas, Shuttleworthia, Desulfosarcina, Melissococcus, Crenobacter, Acidithrix, Methylohalobius and Rheinheimera show a decreased OS in patients, whereas, for protective microbes, higher abundance of Snodgrassella, Tetragenococcus, Marinobacter, Caballeronia, Caenimonas, Dolosigranulum, Amycolatopsis, Olsenella, Alicyclobacillus, Aliagarivorans, Aquamavirus, Thalassobius and Robinsoniella indicated an increased OS (Fig. 2A). Therefore, the microbiome related signature was established with a linear microbiome abundance-based model in which abundance of each genus was weighted by Cox regression coefficient.

Independent value of MRS on prognosis prediction

In TCGA-LIHC microbiome dataset, the 352 patients were divided into high, middle and low groups based on their acquired MRS. Apparently, patients with a higher MRS had a significantly shorter OS ($P < 0.0001$) according to Kaplan-Meier log-rank test (Fig. 2B). Besides, for 1-, 3-, 5-year OS, their AUCs were 0.788, 0.756 and 0.700 respectively. DSS was also found influenced by MRS remarkably (Fig. 2C). Patients with a higher MRS owned a rather shorter OS than these with a lower MRS and the AUCs for 1-, 3-, 5- year DSS were 0.748, 0.709 and 0.658 (Fig. 2D, E).

Serum alpha-fetoprotein (AFP) is widely regarded as a promising biomarker for prognostic stratification(Hu et al., 2022), and we also observed that patients with a positive serum AFP level that is more than 20 ng/ml have a significantly shorter OS (Fig. S1) in this dataset. Previously, we have found that AFP level equals to 400 ng/ml in HCC patients is a pivotal turning point in the transition of molecular characteristics based on (TMT) technology(Wei et al., 2022) and it has an irreplaceable value on the diagnosis and prediction of patients' prognosis. Therefore, we examined whether MRS has an impact on different serum AFP levels by analyzing OS and DSS for AFP > 400 ng/ml and AFP < 400 ng/ml patients with MRS. Interestingly, higher MRS was related to a poor OS and DSS in AFP < 400 ng/ml group ($P < 0.0001$, Fig.S2 A, C), whereas MRS only take an effect on the OS in AFP > 400 ng/ml group ($P < 0.0001$, Fig.S2 B) but no effect its DSS ($P = 0.095$, Fig.S2 D), which indicates that MRS has an AFP-independent value on patients' survival.

To implement the precision medicine in HCC, the identification of molecular subtypes of HCC have recently been researched extensively. For example, Fan Jia group revealed that hepatitis B virus (HBV) related HCC can be classified into three types featured by metabolic reprogramming, microenvironment dysregulation and cell proliferation via integrated multi-omics characterization(Gao et al., 2019) [3]. As for TCGA-LIHC microbiome datasets, we integrated mRNA, miRNA, DNA methylation and CNV information of each sample and stratified them into three subtypes (Fig. 3A). Among those, OS ($P < 0.0001$, Fig. 3B), DSS ($P = 0.0025$, Fig. 3C) and PFS ($P = 0.019$, Fig. 3D) were significantly different and icluster2 type has a poorest prognosis. Subsequent to the further analysis of the impact of MRS on these three subtypes, it was surprisingly found that a high MRS refers to a remarkably poor OS or DSS consistently in all icluster based subtypes (Fig. 4A-F). Moreover, in multivariate Cox regression analysis, we concluded the clinical factors such as gender, age, tumor stage and the condition of vascular invasion as well as the mentioned characteristics including serum AFP level and icluter subtypes on patients' OS and DSS. As we expected, MRS is not a protective factor either in OS (HR = 1.9, $P < 0.0001$, Fig. 5A) or DSS (HR = 1.83, $P < 0.0001$, Fig. 5B). Above evidences implicated that MRS is able to work on patients'

survival independently from not only their clinical factors but also serum and molecular characteristics. By constructing nomogram model with or without MRS (Fig. 6A,C), we assessed its value on prediction of patients' 1-,3- and 5-year survival probability comprehensively, which showed an obvious increase in AUC (without MRS, 1-year AUC:0.756, 3-year AUC:0.688, 5-year AUC:0.681; with MRS, 1-year AUC:0.849, 3-year AUC:0.825, 5-year AUC:0.822).

Potential mechanisms beneath the microbiome in HCC

Given that MRS improved the predictive power of HCC prognosis, it is worthy investigating how the intratumor microbiome influence the biological activity of HCC. By an unsupervised cluster method according to the abundance of 27 microbiome, HCC patients in TCGA can be clustered into three subtypes (Fig. 7A). In C1 cluster, patients mainly possess a high abundance of Aquamavirus, Crenobacter, Thalassobius, Aliagarivorans, Robinsoniella, Melissococcus, Shuttleworthia and Methylohalobius. As for C2 cluster, patients own a high abundance of Olsenella, Candidatus Contendobacter, Amycolatopsis, Alicyclobacillus, Caldimonas, Caballeronia, Pantoea, Acidithrix, Desulfosarcina, Caenimonas and Sediminibacterium. And in C3 cluster, Dolosigranulum, Rheinheimera, Ornithinimicrobium and Roseivirga are the most increased genera (Fig. 7D). Then, we compared OS among these clusters, which reveals that patients in C1 have a best OS while those in C3 have a poorest one (Fig. 7B). However, MRS between C1 and C3 do not have a significant difference unexpectedly (Fig.S3). Since there are tremendous studies reporting that microbiome exerts an effect on cancer immune responses, we predicted each patients' immune response based on TIDE. Though it did not show a significantly variant ratio of immune evasion among clusters, there is still a descending trend of the ratio of immune responders (Fig. 7C). Attempting to explain this phenomenon, we calculated the expression of immune-related molecules and the abundance of infiltrative immune cells with a series of deconvolution methods described before. It reveals that only naïve B cells, follicular helper T cells and M1 type macrophages are variant while the abundance of other cellular types such as CD8 T cells remain almost the same (Fig. 7E). And for immune molecules, we defined CD274, CTLA4, PD1, CD160, LAG3, IDO1, HAVCR2 as immune suppressive markers and CXCL10, CXCL9, IFN-γ, TBX2, GZMA, GZMB, PRF1 and CD8a as immune active markers. It uncovers that HCC microbiome-related subtypes have a distinct expression of CD274, CD160, IDO1, HAVCR2, CXCL10, CXCL9 and PRF1 (Fig. 7F). In total, the immune activation maybe more powerful than immune suppression in C1 cluster but C3 cluster may own an opposite status. Interestingly, both in abundance of immune infiltrative cells and expression of immune related molecules, C1 and C3 cluster have a higher mean level than C2 cluster, which is consistent with their MRS but cannot explain their differences in OS completely.

Since C3 cluster patients acquire the least benefit from immunotherapy and the poorest survival, we subsequently employed WGCNA method to mine crucial gene modules of this subtype in TCGA-LIHC microbiome dataset. Initially, we set the optimal soft-threshold power at 6 (Fig. 8A) and the least gene numbers in each module at 30. Then genes with similar expression were clustered into 8 modules (Fig. 8C). Among these 8 modules, the turquoise module showed the strongest positive correlation with C3 cluster (ME = 0.12, P = 0.02) and the most negative association with C2 cluster (ME = 0.11, P = 0.05) at the same time (Fig. 8D). Thus, the turquoise module was selected to perform further analysis with the criteria of MM > 0.8 and GS > 0.1 to filter 127 hub genes (Fig. 8E, F). Then we employed KEGG pathway enrichment analysis in the turquoise module and the result revealed that one of the principally enriched pathways was termed "Signaling pathways regulating pluripotency of stem cells" (Fig. 8G), which partly indicated that intratumor microbiome possibly affect the

stemness of tumor cells. Hence, we calculated 26 reported stemness gene sets score in each HCC sample by ssGSEA algorithm, in which 9 stemness gene sets were significantly different among the three clusters (Fig. 8H). Compared with their mean scores, the trend is similar to their OS, which may finally elucidate the assumption mentioned above.

Discussion

The microbiota plays an important role in human health and diseases(Xia et al., 2023; Xiang et al., 2023). In recent years, Organs and tissues traditionally regarded as sterile have been found to contain different microbial populations, and these microbial populations play a vital role(Xue et al., 2023). Many studies have found that intratumor microbiome matters in the response to tumor microenvironment, which may influence the prognosis of patients(Y. Huang et al., 2004; Qu et al., 2022). Nevertheless, the relationship between the prognosis of HCC patients and intratumor microbiome has not been deeply discussed. Hence, this study aimed to explore the promising prognostic predictors through investing the HCC intratumor microbiome signature, which will provide new insights into the prognosis of HCC patients.

Firstly, the microbiome related model for HCC was successfully constructed. Through the univariate and multivariate Cox analyses, 27 microbes were identified to have an independent effect on the OS of HCC patients. Both prognosis risk and protective microbes were included. Several microbes have been found to play a vital role in cancers. For example, the abundance of Sediminibacterium was found to be important in both gastric cancer and lung cancer(Cheng et al., 2020; Nikitina et al., 2023), and the abundance of Tetragenococcus also matters in oral cancer(Guo et al., 2020). The MRS model for HCC was further established. Based on the acquired MRS, it was found that HCC patients with higher MRS owned shorter OS and DSS than those with lower MRS, which showed that the established MRS model can greatly predict the prognosis of patients with HCC.

It was also demonstrated that AFP stratification will not affect the prognosis prediction ability of the established MRS model although AFP is considered a prognostic biomarker for HCC patients(Johnson et al., 2022). Besides, HCC patients with a high MRS can have poor OS and DSS regardless of icluster based on subtypes. Furthermore, MRS model was found to be an independent risk factor for the prognosis of HCC patients. Hence, we can conclude that MRS can effectively predict the OS and DSS of HCC patients independent of not only their clinical factors but also serum and molecular characteristics.

So far, a large amount of biomarkers have been identified to predict the response to immunotherapy in HCC(Lin et al., 2022; Shen et al., 2020). Nejman et al. found that different types of intratumor bacteria are mainly in cancer and immune cells, which further implied that intratumor microbiome may have a close correlation with tumor immune characteristics(Nejman et al., 2020). Therefore, microbiome may shed light on the future perspective of biomarkers for HCC immune therapy. By an unsupervised cluster method according to the abundance of 27 microbiome, enrolled HCC patients can be clustered into three subtypes, including C1, C2 and C3. C1 cluster has better OS compared to C3 cluster although there exists no significant difference in MRS. We further explored the expression of immune-related molecules and the abundance of infiltrative immune cells, and we found that the immune activation in C1 cluster is more powerful than suppression but C3 cluster is opposite. However, both C1 and C3 cluster have a higher mean level of immune infiltrative cells and related

molecules than C2 cluster, which is consistent with the trend of MRS. This cannot explain differences in OS. Furthermore, the enriched pathways called “signaling pathways regulating pluripotency of stem cells” was found, and we explored the relationship between the stemness of tumor cells and intratumor microbiome. 9 stemness gene sets were found to significantly affect intratumor microbiome. Through comparing their mean score, we demonstrated that it was consistent with OS. Hence, the above findings further showed that there exists a close correlation between the stemness of tumor cells and intratumor microbiome.

Conclusion

A 27 intratumor microbiome prognostic signature named MRS was established successfully as a distinct model for predicting prognosis of HCC patients. Moreover, MRS exerted an independent effect on OS and DSS on clinical factors, pathological factors and multi-omics based molecular subtypes. Besides, we investigate potential mechanism of intratumor microbiome and it implicated that intratumor microbiome mainly influence the prognosis of HCC via modulating cancer stemness. Furthermore, interventions targeting microbiome raises a possibility for HCC patients to have a better prognosis.

Abbreviations

HCC: hepatocarcinoma

OS: overall survival

DSS: disease-specific survival

WGCNA: weighted correlation network analysis

GSVA: gene set variation analysis

MRS: microbiome-related score

AUC: area under the ROC curve

ROC: receiver-operating characteristic

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yisu Song. The first draft of the manuscript was written by Yisu Song and Ze Xiang, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

Data availability

All data used in this study are available on the cBioportal and UCSC Xena website.

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Figures

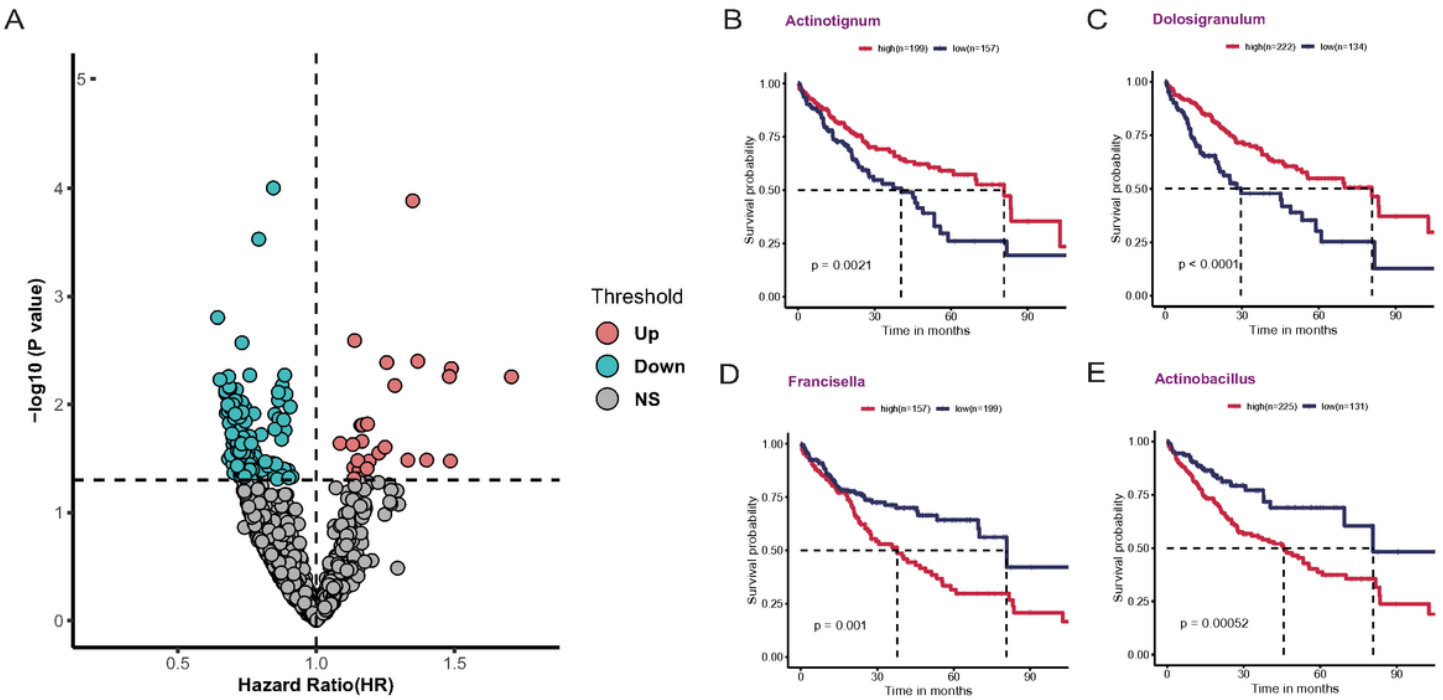

Figure 1

Relationships between microbial abundance and OS by univariate Cox regression analysis. A. The association between microbial abundance and OS is displayed with a volcano plot. B-E. Kaplan-Meier OS curves based on the abundance Actinotignum, Dolosigranulum, Francisella and Actinobacillus in HCC patients are taken as examples. The patients are divided into a high or low group according to their individual microbial abundance. The p values of differences between two groups are calculated by log-rank test. OS, overall survival; HCC, hepatocellular carcinoma.

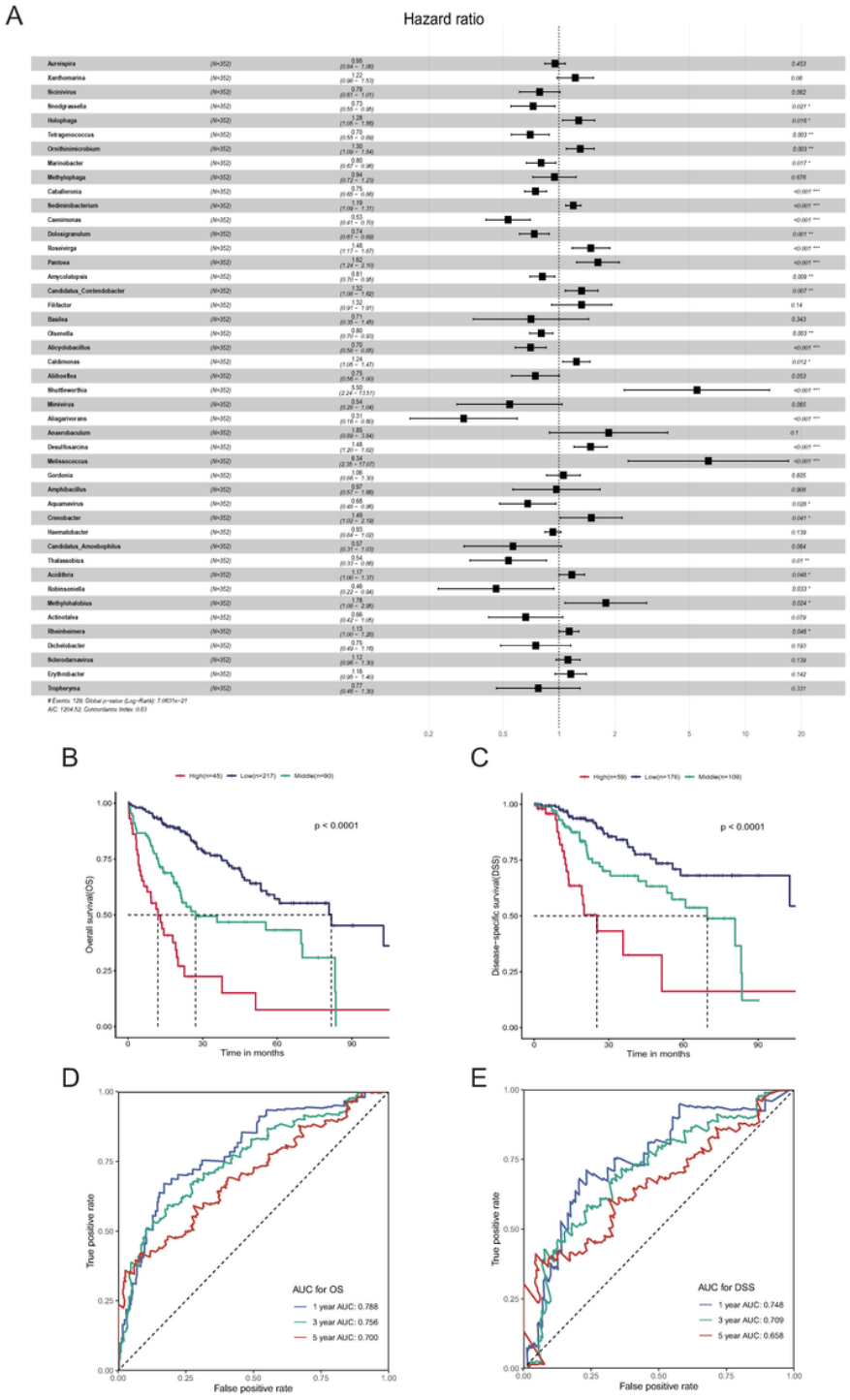

Figure 2

Impact of MRS on OS and DSS. A. The hazard ratio of part candidates and the final microbiome signature obtained from multivariate Cox regression analysis on patients' OS were shown in the forest plot. B-C. Kaplan-Meier curves of OS and DSS in HCC patients are illustrated according to MRS. E-F. ROCs are plotted to elucidate the performance of MRS on predicting OS and DSS. The p values are calculated by log-rank test. *P < 0.05, **P < 0.01, ***P < 0.001.

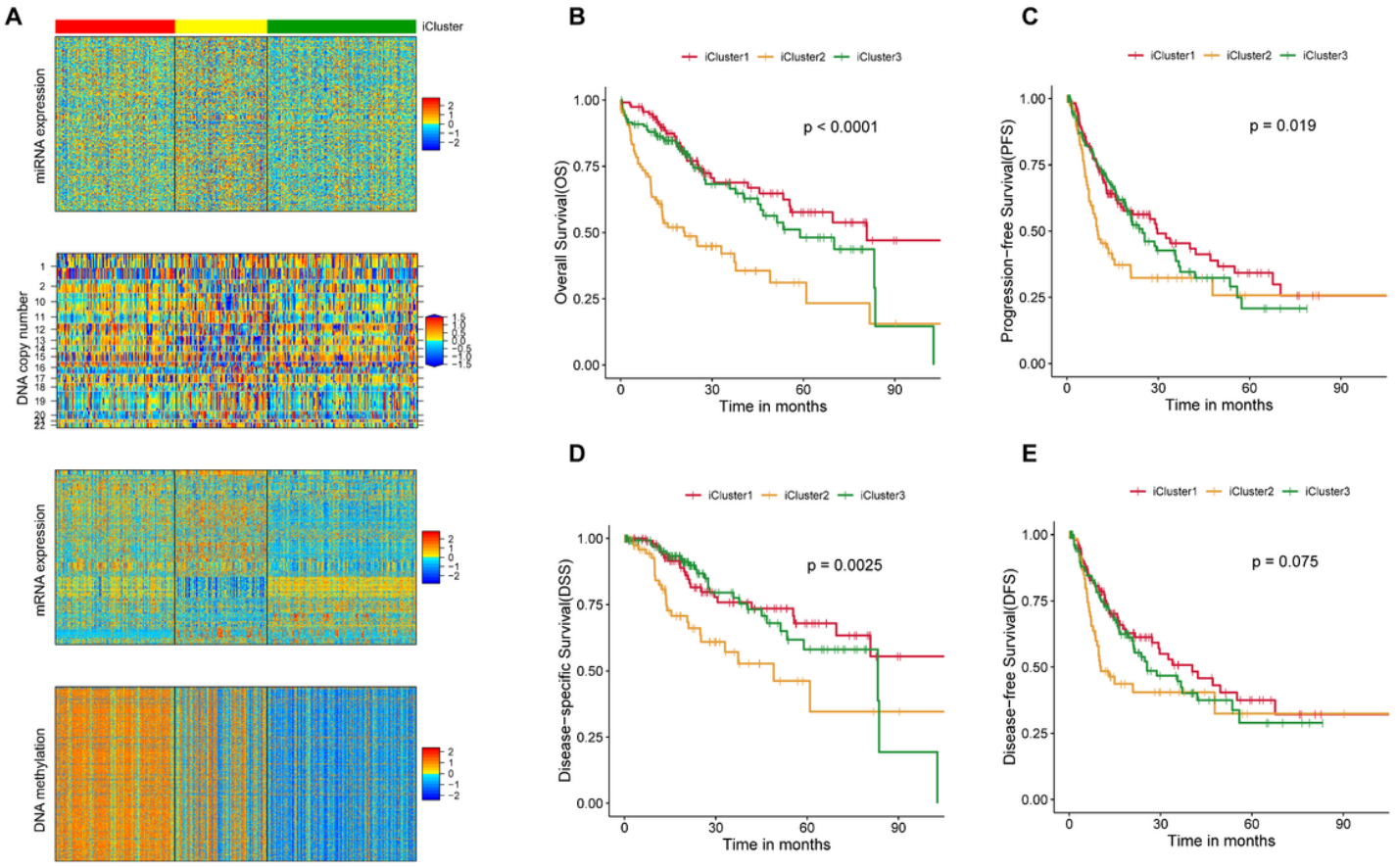

Figure 3

Molecular subtypes of HCC with integrated multi-omics and their differences on OS, DSS, PFS and DFS. A. Molecular subtypes of HCC based on miRNA expression, DNA copy number, mRNA expression and DNA methylation are identified by icluster algorithm into 3 clusters. B-E. Kaplan-Meier curves of OS, DSS, DFS and PFS of these three clusters are plotted. The p values are calculated by log-rank test.

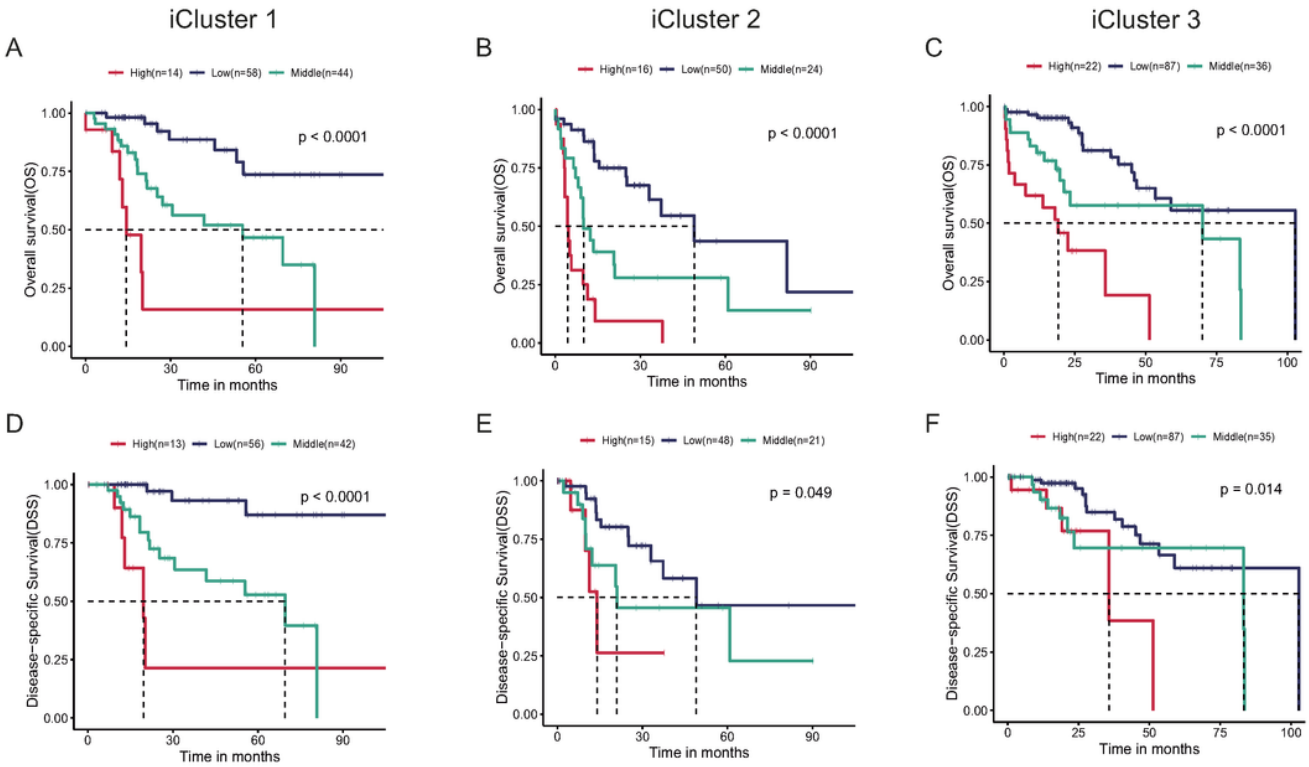

Figure 4

Effect of MRS within icluster based molecular subtypes on OS and DSS. A-C. Kaplan-Meier OS (top panel) curves for HCC in three icluster subtypes are presented according to the level of MAPS. D-F. Kaplan-Meier DSS (bottom panel) curves for HCC in their corresponding icluster are presented. The patients were divided into high, middle, and low groups. The p values shown were calculated by log-rank test among three groups

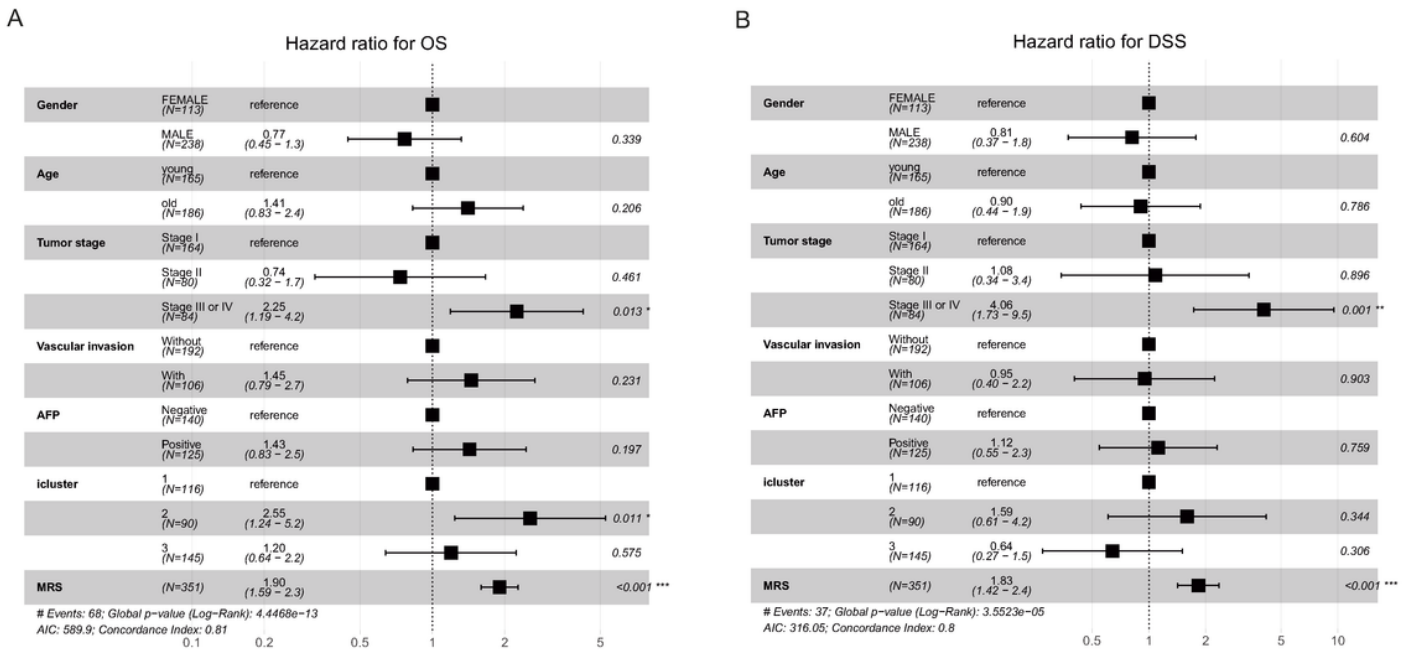

Figure 5

Independent effect of MRS on OS and DSS. A-B The forest plot illustrates the hazard ratio of clinical factors, pathological factors, molecular subtype and MRS analyzed by multivariate Cox regression for OS and DSS respectively.

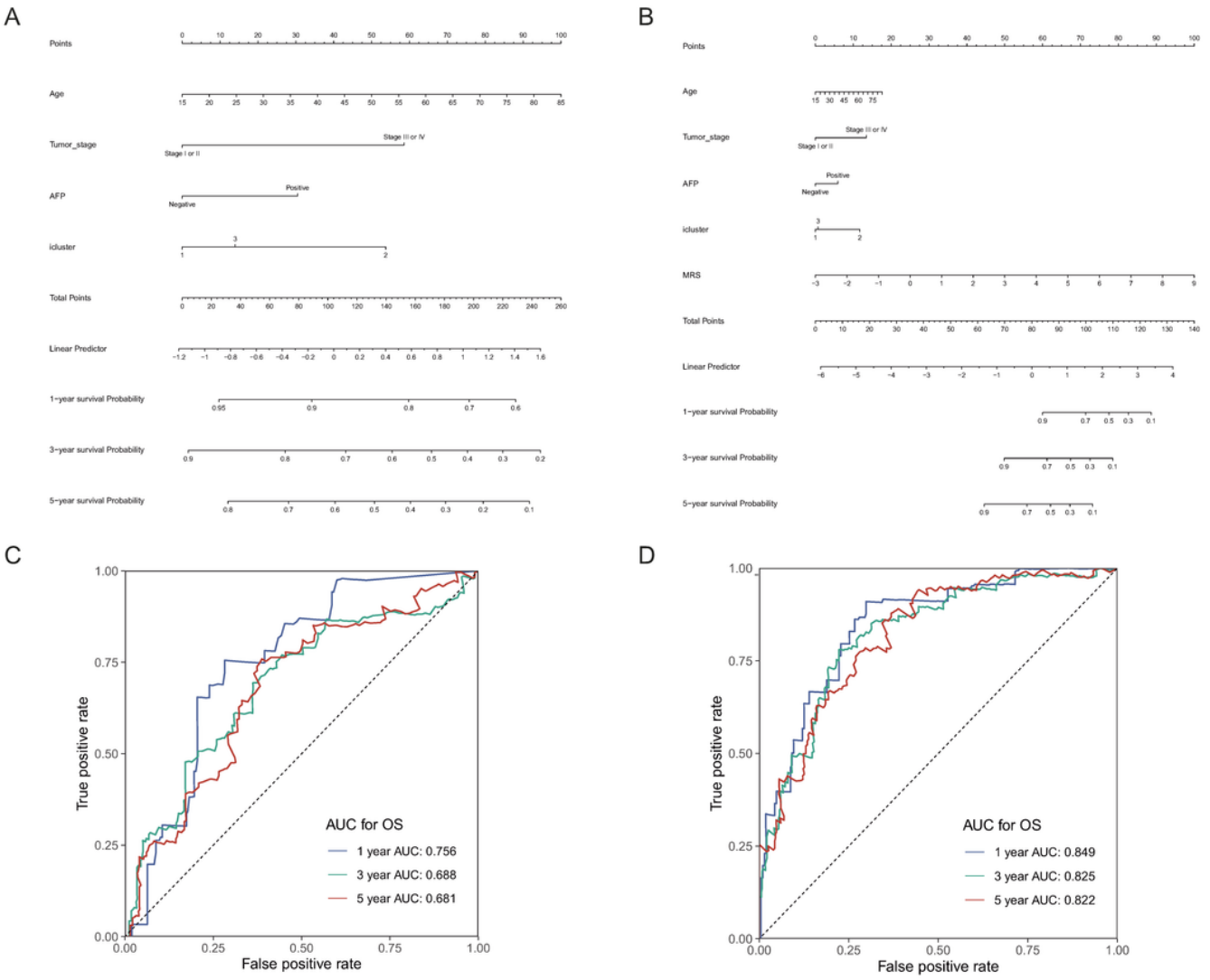

Figure 6

Nomograms with or without MRS are displayed to predict the 1-, 3-, and 5-year OS of HCC patients. A-B. Nomograms are developed based on the integration of clinical factors, pathological factors and molecular subtypes without and with MRS respectively. C-D. The corresponding ROCs elucidate the performance of nomogram model without and with MRS.

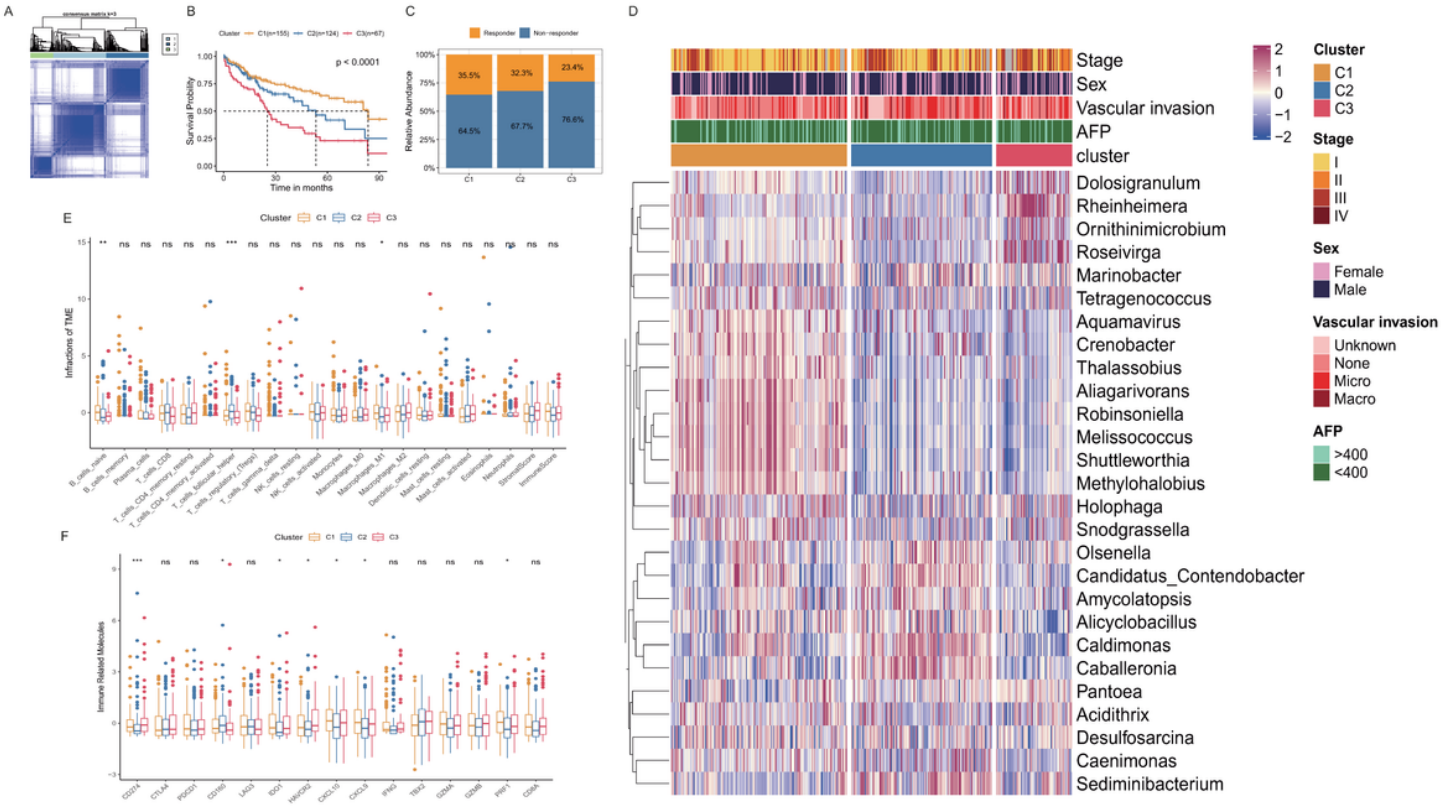

Figure 7

Intratumor microbiome abundance-based clusters of HCC with their immune characteristics. A. Consensus clustering identified three clusters of HCC with different intratumor microbiome abundance in 27 microbiome-related signatures. B. Kaplan–Meier OS curves for HCC patients among distinct clusters. C. The percents of responder and non-responder to immunotherapy among microbiome subtype-based clusters estimated by TIDE algorithm. D. Heatmap illustrates the landscape of intratumor microbiome abundance of 27 microbiome-related signature in three clusters. E. Box plot displays the differences of 22 infiltrating immune cells, stromal and immune scores among the three clusters by Kruskal–Wallis test. F. Box plot are presented about the differences of immune suppressive and activated molecules expression among the three clusters by Kruskal–Wallis test. ns, not significance, *P < 0.05, **P < 0.01, ***P < 0.001.

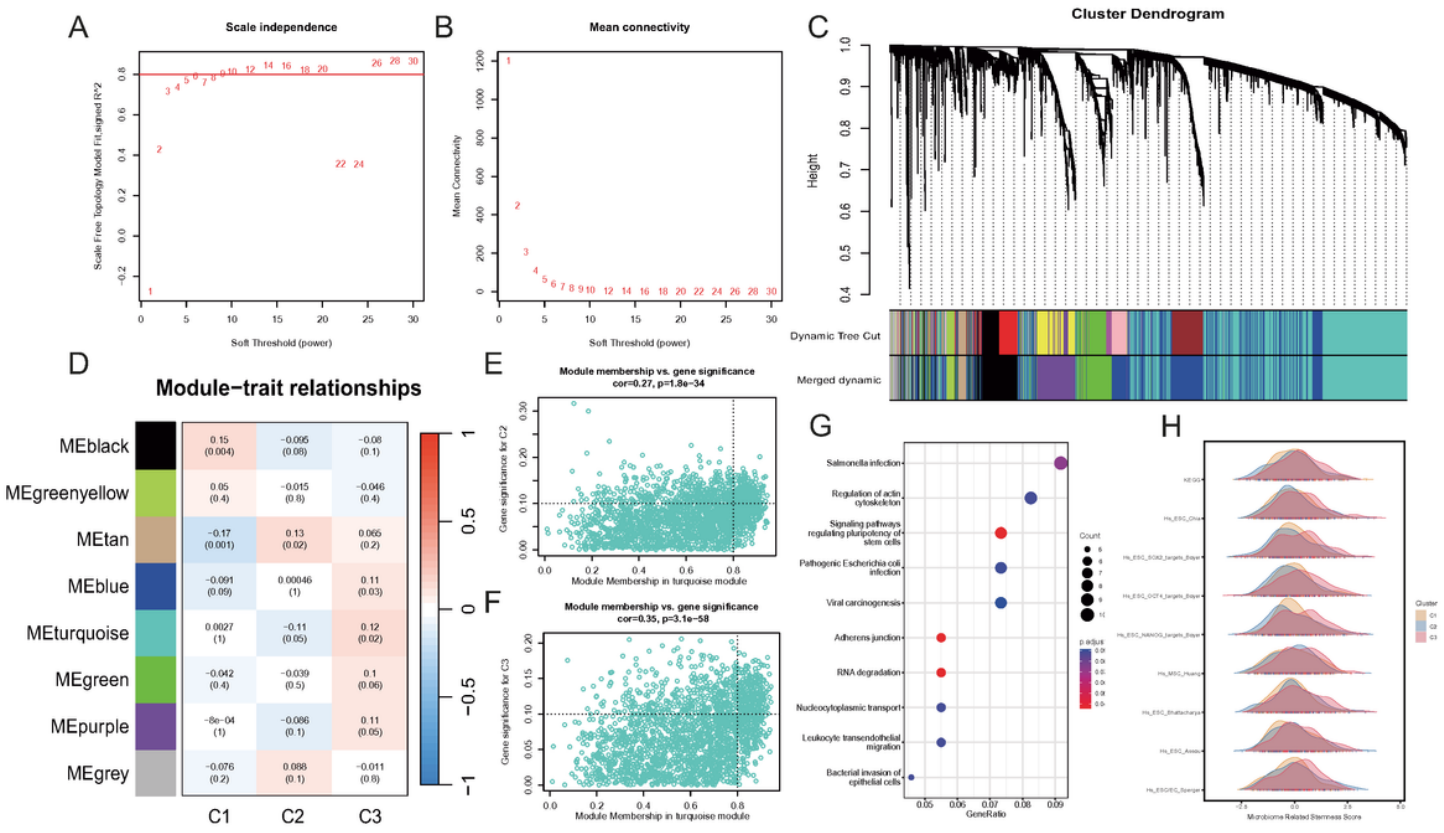

Figure 8

Identification of hub genes by WGCNA for HCC intratumor microbiome subtypes with their function enrichment. A-B. Scale independence and mean connectivity of multiple soft-thresholding powers from 1 to 30. C. The cluster dendrogram was established with the weighted correlation coefficients and clustered co-expression genes with similar expression characteristics, each of which represents a module. D. Heatmap displays the correlation between module eigengenes and clinical traits as well as intratumor microbiome-related subtypes. E-F Scatter plot depicts association of gene significance for Cluster C2 and Cluster C3 with module membership in turquoise module. G. KEGG analysis of intersectant genes of C2 and C3 associated module. H. Ridge plot of 9 gene sets concerning cell stemness with significant difference calculated by Kruskal–Wallis test.

Supplementary Files

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