

Herbal Medicine in the Molecular Era: Integrating Traditional Chinese Herb Signatures with Chemotherapy Efficacy and Tumor Microenvironment Using Machine Learning Models

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Abstract. Traditional Chinese Medicine (TCM) offers a rich repertoire of bioactive compounds whose molecular targets can now be mapped systematically against highthroughput cancer data. Here, we combine networkbased herb–gene associations with machine and deep learning to both predict therapeutic response and elucidate tumor microenvironment (TME) modulation in metastatic melanoma. First, we derived regulatory signatures for three herbs—WuHuaGuo (Fig), JiuCengTa (Basil), and Codonopsis pilosula (DangShen)—and mapped their putative gene targets onto TCGA melanoma transcriptomes. Kaplan–Meier and Cox analyses confirmed that high herb regulatory scores correlate with improved overall survival and enriched immune infiltration. Next, we trained four classifiers (logistic regression, random forest, knearest neighbors, and neural network) on GDSC cellline expression and logIC₅₀ data to predict chemosensitivity using each herb’s signature. Nonlinear models (RF, NNET) achieved robust discrimination (AUC > 0.8) for DNA damage–targeting (PARP) and epigenetic (HDAC) inhibitors, highlighting the power of ensemble and deeplearning approaches to capture complex, nonlinear patterns in herbreregulated pathways. To validate our in silico predictions, we treated B16F10 melanoma cells with Fig and Basil extracts, observing dosedependent growth inhibition (distinct IC₅₀ values) and, in coculture assays, significant upregulation of Tcell activation markers CD44 and CD69. Together, these results showcase a unified computational–experimental framework that leverages TCM herb signatures and modern machine learning to forecast drug response and enhance antitumor immunity, offering a blueprint for discovering novel adjuvant therapies in melanoma.

Keywords: melanoma, machine learning, herb, antitumor immunity

1. Introduction

Melanoma, arising from the malignant transformation of melanocytes, is the most aggressive form of skin cancer, responsible for over 80% of skin cancer-related deaths despite accounting for only 4–5% of cases [1,2]. Its lethality stems from rapid metastasis to vital organs such as the liver, lungs,

and brain, with a 5-year survival rate dropping to 16% in advanced stages [3]. While surgical resection remains effective for early-stage lesions, metastatic melanoma (MM) resists conventional therapies like chemotherapy and radiotherapy due to non-specific cytotoxicity and acquired drug resistance [1].

The introduction of immune checkpoint inhibitors (ICIs), such as anti-PD-1 and anti-CTLA-4 antibodies, revolutionized therapy by reinvigorating exhausted T cells to attack tumor cells [4]. Recent long-term follow-up data from the CheckMate 067 trial further solidify this promise. In this pivotal study, with a minimum follow-up of 10 years, patients treated with nivolumab (anti-PD1) plus ipilimumab (anti-CTLA4) had a median overall survival of 71.9 months, compared with 36.9 months with nivolumab monotherapy and 19.9 months with ipilimumab monotherapy [5]. Moreover, the combination therapy achieved a median melanoma-specific survival exceeding 120 months—with 37% of patients alive at trial completion—and among patients who were progression-free at 3 years, the 10-year melanoma-specific survival rates were an impressive 96% for the combination regimen [5]. Immunotherapy is really the game changer for MM. Despite these breakthroughs, many patients still exhibit primary resistance or eventually develop acquired resistance to ICIs due to complex mechanisms—including altered antigen presentation, an immunosuppressive tumor microenvironment (TME), and chronic T cell exhaustion [6]. This dual challenge of melanoma's intrinsic aggressiveness and its ability to evade even the most advanced immunotherapies underscores the pressing need for new, multifaceted treatment approaches.

Traditional Chinese Medicine (TCM), with its 3,000-year history, offers a treasure trove of bioactive compounds that modulate cancer biology through polypharmacological mechanisms. Chinese herbal medicine (CTMH) lies at the heart of TCM and has been traditionally used to restore balance in the body through natural remedies. Early studies demonstrated that specific Chinese herbs and herbal formulations could inhibit tumor growth and metastasis via inducing apoptosis of tumor cells [7]. For instance, curcumin has been shown to induce apoptosis, inhibit cell proliferation, and modulate various signaling pathways such as NF- κ B, Wnt, and PI3K/Akt in cancer cells [8,9]. Berberine, extracted from the Berberis plant, has shown potential in inducing cell cycle arrest and apoptosis, as well as enhancing the sensitivity of cancer cells to conventional treatments [10]. Beyond these, other herbal compounds like dioscin, baicalein, wogonin, and silibinin have been identified for their anticancer activities via anti-proliferative, pro-apoptotic, anti-metastatic, and anti-angiogenic effects [11-13]. Furthermore, the integration of CTMH with conventional cancer treatments, such as chemotherapy and radiotherapy, has shown promise in reducing side effects, improving patient quality of life, and enhancing the overall effectiveness of treatment [14,15].

However, while these findings have been promising, they have been limited to a handful of well-characterized compounds and small patient cohorts. To date, the majority of studies have not provided a comprehensive analysis across hundreds of TCM herbs nor have they integrated a detailed analysis of the tumor microenvironment. In other words, despite evidence that many herbal remedies can regulate processes such as cell proliferation, apoptosis, adhesion, migration, and even immune cell recruitment, no study has yet mapped these effects on a pan-cancer level across large-scale datasets. This gap means that our current understanding remains fragmented, lacking a systematic evaluation of how these herbs affect tumor biology on a molecular network level and within the context of a complex and dynamic TME.

Therefore, in this project we seek to address these limitations by employing a computational framework that integrates high-throughput transcriptomic data from The Cancer Genome Atlas (TCGA) [16] with a curated TCM herb-gene interaction database. Recent advances in network

medicine have provided a paradigm to decode TCM's polypharmacology. Gan et al. (2023) established that herb effectiveness correlates with the network proximity of their protein targets to disease-associated gene modules in the human interactome [17]. In our study, we applied Gene Set Variation Analysis (GSVA) to compute herb regulatory scores for hundreds of herbs across a metastatic melanoma patient cohort from TCGA [18]. For each herb, patients were stratified into high versus low enrichment groups based on the expression levels of herb-regulated genes. We then generated comprehensive survival and immune profiles, as well as assessed mutation burdens and structural variations, across all herbs. Our systematic screening revealed that, among all the herbs, Fig (WuHuaGuo), Basil (JiuCengTa), and Codonopsis pilosula (DangShen) were the top candidates associated with the best overall survival and the most favorable immune features—most notably, a higher level of tumor-infiltrating T cells. To validate these computational predictions experimentally, we extracted active compounds from the three top-ranked herbs and assessed their cytotoxic effects on B16F10 melanoma cells. In vitro assays demonstrated dose-dependent growth inhibition, with distinct IC50 values observed for each extract. Furthermore, flow cytometric analysis of T cell activation in vitro showed that treatment with Fig or Basil extracts significantly upregulated T cell activation markers CD44 and CD69, suggesting that these herbs enhance T cell activation and contribute to an anti-tumor immune response.

Taken together, our integrative analysis—combining comprehensive computational screening of herb-regulated gene signatures across all patient samples with rigorous experimental validation—demonstrates that select TCM herbs can remodel the tumor microenvironment and potentially improve patient outcomes in metastatic melanoma. By bridging ancient pharmacopeia with 21st-century computational biology, this work advances TCM's role in precision immuno-oncology, offering a blueprint for repurposing natural compounds against therapy-resistant cancers.

2. Methods

2.1. Learning R and setting up the computational environment

R programming was utilized to set up the computational environment necessary for this study. Fundamental packages required for computational biology analysis were installed, including GSVA for gene set variation analysis, survminer for survival analysis, and dplyr and tibble for data handling. BiocManager was installed to manage Bioconductor packages essential for large-scale genomics datasets. The TCGA RNA-seq dataset was downloaded from the Firehose repository, specifically focusing on Skin Cutaneous Melanoma (SKCM) samples. Given the high variability in raw RNA-seq data, a log10 transformation was applied to normalize gene expression levels, minimizing noise and ensuring consistency across samples. The dataset was validated for accuracy and completeness before proceeding with further analysis.

2.2. Gene Set Variation Analysis (GSVA) for herb regulatory score calculation

GSVA was employed to evaluate the influence of Chinese Traditional Medicine Herbs (CTMH) on melanoma prognosis by computing a regulatory score for each herb in each patient. The HIT herb target dataset, which maps herbs to their known target genes based on network pharmacology studies, was utilized. Gene-herb associations from a referenced study were used to construct a gene set list, grouping genes by Herb ID for GSVA input. The SKCM RNA-seq dataset was processed using GSVA to generate herb regulatory scores for each patient, which were stored in an .Rda file

for further analyses. The GSVA computation took approximately 10 minutes, and the dataset was validated to ensure the presence of regulatory scores for all samples.

2.3. Survival analysis of herb regulatory scores

Kaplan-Meier survival analysis and Cox proportional hazards regression were conducted to examine the correlation between herb regulatory scores and melanoma patient survival using the TCGA melanoma cohort. Clinical metadata was processed to extract key survival variables, including days to death, days to last follow-up, and patient vital status. Patients with incomplete survival records were filtered out to ensure reliability. Time-to-event variables were standardized in years, integrating death events and censoring data. GSVA-derived herb regulatory scores were matched with clinical survival data, and patients were categorized into high-score and low-score groups based on median herb expression levels. Kaplan-Meier survival analysis was performed, followed by log-rank tests for statistical significance assessment. Cox proportional hazards regression was conducted to estimate hazard ratios (HRs) and determine whether higher herb scores were associated with improved prognosis.

2.4. Tumor immune landscape analysis

The relationship between herb regulatory scores and immune cell infiltration in melanoma tumors was examined using the TCGA immune landscape dataset. Spearman correlation analysis was conducted to assess associations between herb scores and immune infiltration metrics. Herbs strongly associated with immune infiltration were compared with those linked to positive patient survival. The three most relevant herbs—Jiu Ceng Ta, Dang Shen, and Wu Hua Guo—were identified based on their associations with immune landscape features and favorable prognosis. Correlation analyses revealed strong positive relationships between these herbs' regulatory scores and increased T-cell and B-cell receptor diversity, suggesting their potential role in enhancing immune surveillance. Additionally, patients with high herb regulatory scores exhibited increased leukocyte fractions, indicative of an immune-active tumor microenvironment.

2.5. Genomic instability and herb regulatory scores

To investigate the potential influence of herbs on tumor progression, GSVA-derived herb regulatory scores were correlated with genomic instability features, including tumor mutation burden (TMB), copy number variations (CNVs), and homologous recombination deficiency (HRD). TCGA-derived genomic instability metrics were integrated, and correlation analyses were conducted. The three identified herbs—Jiu Ceng Ta, Dang Shen, and Wu Hua Guo—were consistently associated with improved patient survival and stronger immune responses. Additionally, they correlated with genomic stability, demonstrating lower mutation burdens, reduced chromosomal alterations, and enhanced DNA repair mechanisms.

2.6. Drug sensitivity prediction using machine learning and deep learning models

We utilized the GDSC_2020(v2-8.2) pharmacogenomic dataset (downloaded via PharmacoGx) to predict ovarian cancer cell-line drug responses based on three pre-defined gene signatures. Raw RNA-seq expression (Kallisto_0.46.1) and IC₅₀ sensitivity profiles were extracted with median-of-replicates summarization, and IC₅₀ values were log-transformed. Drugs and cell lines exhibiting >20% missing sensitivity measurements were excluded. Gene-level expression was mapped from

Ensembl IDs to HGNC symbols via biomaRt, collapsing duplicate symbols by maximum variance. Genes regulated by each herb were iteratively subsetted to the mapped expression matrix; for each signature, cell lines with >20% missing signature expression were removed and the corresponding sensitivity profiles were aligned. Predictive modeling employed four classifiers—logistic regression (GLM), random forest (RF), k-nearest neighbors (KNN), and neural network (NNET)—within a repeated 5-fold cross-validation framework (3 repeats), optimizing ROC AUC via the caret package with class probabilities and two-class summary metrics. Near-zero-variance predictors were discarded, and RF/KNN/NNET models were centered and scaled prior to training; RFs used 500 trees, and NNETs allowed up to 5,000 weights. For each drug–signature combination, we recorded AUC, number of cell lines, and dominant tissue of origin (from GDSC metadata). To visualize performance, we generated PDF figures (via pdf()/gridExtra) of: (1) AUC distribution bars for models with AUC > 0.8; (2) AUC versus sample size scatterplots; (3) tissue-stratified heatmaps of high-AUC results; (4) boxplots of AUC by drug class; and (5) combined summary grids juxtaposing top-drug bars and model-comparison boxplots. All code and output files are available in the project repository.

2.7. Melanoma cell growth assay

To assess the direct anti-tumor effects of herbal extracts on melanoma cells, an in vitro growth assay using B16F10 melanoma cells was conducted. B16F10 cells were cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified incubator with 5% CO₂ and subcultured upon reaching 70-80% confluency.

For treatment, cells were seeded at 5,000 cells per well in 96-well plates and allowed to adhere overnight. Serial dilutions of Jiu Ceng Ta, Dang Shen, and Wu Hua Guo extracts were prepared in DMSO, ensuring final concentrations of 0.02 mg/mL, 0.1 mg/mL, 1mg/mL, 5 mg/mL, and 10 mg/mL. The culture media was replaced with media containing the respective herbal extracts or DMSO (control). After 48 hours, cell viability was assessed using the CCK8 reagent, with absorbance measured at 450 nm. Morphological changes were observed under a microscope to evaluate cytotoxic effects.

2.8. Immune cell activation and cytotoxicity assays

To assess the immune-modulatory effects of herbal extracts, mouse peripheral blood mononuclear cells (PBMCs) were obtained from a commercial supplier and cultured in RPMI-1640 medium supplemented with 10% FBS, 1% penicillin-streptomycin, and 50 µM β-mercaptoethanol. T cells were activated in vitro using anti-CD3/CD28 stimulation for 48 hours before herbal extract treatment. The same herbal extract concentrations used in the melanoma cell growth assay were applied to the T cell cultures. Following treatment, T cell activation was analyzed by flow cytometry, assessing CD69, CD44, and CD62L expression. Viability staining with DAPI was performed to confirm cell survival.

2.9. Statistics

All statistical analyses were performed in R (version 4.3.2). For survival analysis, Kaplan-Meier curves were generated with survival probabilities compared via the log-rank test, and Cox proportional hazards regression was used to estimate hazard ratios (HRs) and confidence intervals.

Spearman correlation analysis was employed to investigate associations between herb regulatory scores and immune or genomic metrics. Comparisons of cell viability and T cell activation between treatment groups were made using one-way ANOVA with post hoc Tukey's multiple comparisons test or unpaired two-tailed Student's t-tests, as appropriate. All data are presented as mean $\pm$ standard error (SE) unless otherwise noted. p-values below 0.05 were considered statistically significant.

3. Results

3.1. Kaplan-meier survival analysis demonstrating improved prognosis with high Basil, Fig, and DangShen regulatory scores in the TCGA melanoma cohort

After an initial computational screening of a broad panel of Traditional Chinese Medicine (TCM) herbs in the TCGA melanoma cohort, we identified 64 candidate herbs with hazard ratios (HR) below 1 and p-values below 0.05 (Supplementary Table 1). From this subset, Basil (JiuCengTa), Fig (WuHuaGuo), and Codonopsis pilosula (DangShen) emerged as top-ranking candidates, demonstrating not only statistically significant survival benefits but also strong correlations with active immune responses, including elevated tumor-infiltrating lymphocyte (TIL) scores. Notably, these three herbs are also frequently encountered in everyday life, whether as dietary supplements or common foods, highlighting their broad accessibility and potential translational impact.

As shown in Figure 1A, patients stratified into the high Basil regulatory score group exhibited significantly prolonged overall survival relative to those with lower Basil scores ($p = 0.0045$). Similarly, increased Fig ($p = 0.038$; Figure 1B) and DangShen ($p = 0.008$; Figure 1C) regulatory scores were associated with improved patient outcomes. These findings further substantiate the notion that certain TCM herbs can favorably modulate disease progression, potentially by orchestrating enhanced immune cell infiltration and immune surveillance within the tumor microenvironment.

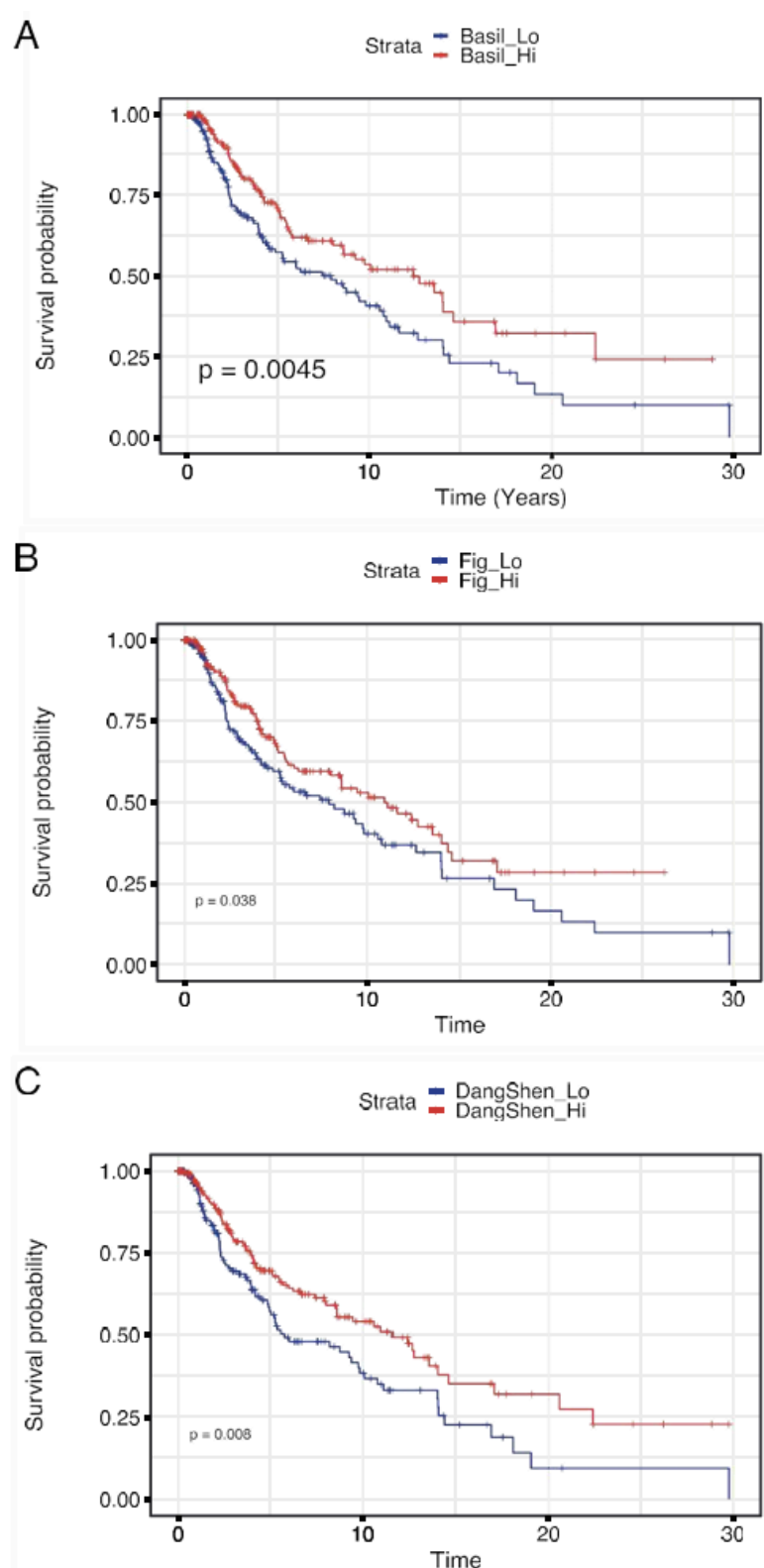


Figure 1. Kaplan-Meier survival analysis of top-ranked TCM herbs in TCGA melanoma cohort

Kaplan-Meier survival curves depict the prognostic impact of GSVA-derived regulatory scores for Basil (JiuCengTa), Fig (WuHuaGuo), and DangShen (Codonopsis pilosula). In each panel (A–C), patients were stratified into high (red line) versus low (blue line) regulatory score groups based

on the median value. Log-rank tests showed that elevated scores for each herb were significantly associated with prolonged overall survival, highlighting the potential role of these TCM herbs in improving clinical outcomes in metastatic melanoma.

3.2. Elevated herb regulatory scores are associated with enhanced lymphocyte infiltration and IFN- γ response signatures

Having identified Basil (JiuCengTa), Fig (WuHuaGuo), and Codonopsis pilosula (DangShen) as top candidates linked to survival benefits, we next examined whether high regulatory scores for these herbs also correlated with markers of an active anti-tumor immune response. As shown in Figure 2A, patients with high Basil regulatory scores displayed markedly increased lymphocyte infiltration signature scores ($p = 9.78 \times 10^{-40}$), alongside higher IFN- γ response levels ($p = 1.02 \times 10^{-21}$) when compared with their low-score counterparts. A similar pattern emerged for Fig (Figure 2B), where elevated regulatory scores were associated with significantly enhanced lymphocyte infiltration ($p = 2.18 \times 10^{-27}$) and a stronger IFN- γ response ($p = 2.81 \times 10^{-15}$). Likewise, DangShen (Figure 2C) exhibited robust correlations with both heightened lymphocyte infiltration ($p = 1.89 \times 10^{-9}$) and elevated IFN- γ activity ($p = 1.4 \times 10^{-18}$).

The enrichment of tumor-infiltrating lymphocytes (TILs) is well recognized as a favorable prognostic factor in multiple cancer types, including melanoma, as it often signifies a more robust immune surveillance capable of targeting malignant cells [19,20]. Moreover, IFN- γ is a key immunomodulatory cytokine that activates macrophages, enhances antigen presentation, and supports T cell-mediated cytotoxicity, thus playing a central role in shaping effective anti-tumor immunity [21,22]. Consequently, the strong correlation of Basil, Fig, and DangShen regulatory scores with higher TIL infiltration and IFN- γ response underscores the immunostimulatory potential of these herbs, offering mechanistic insight into their observed survival benefits in metastatic melanoma.

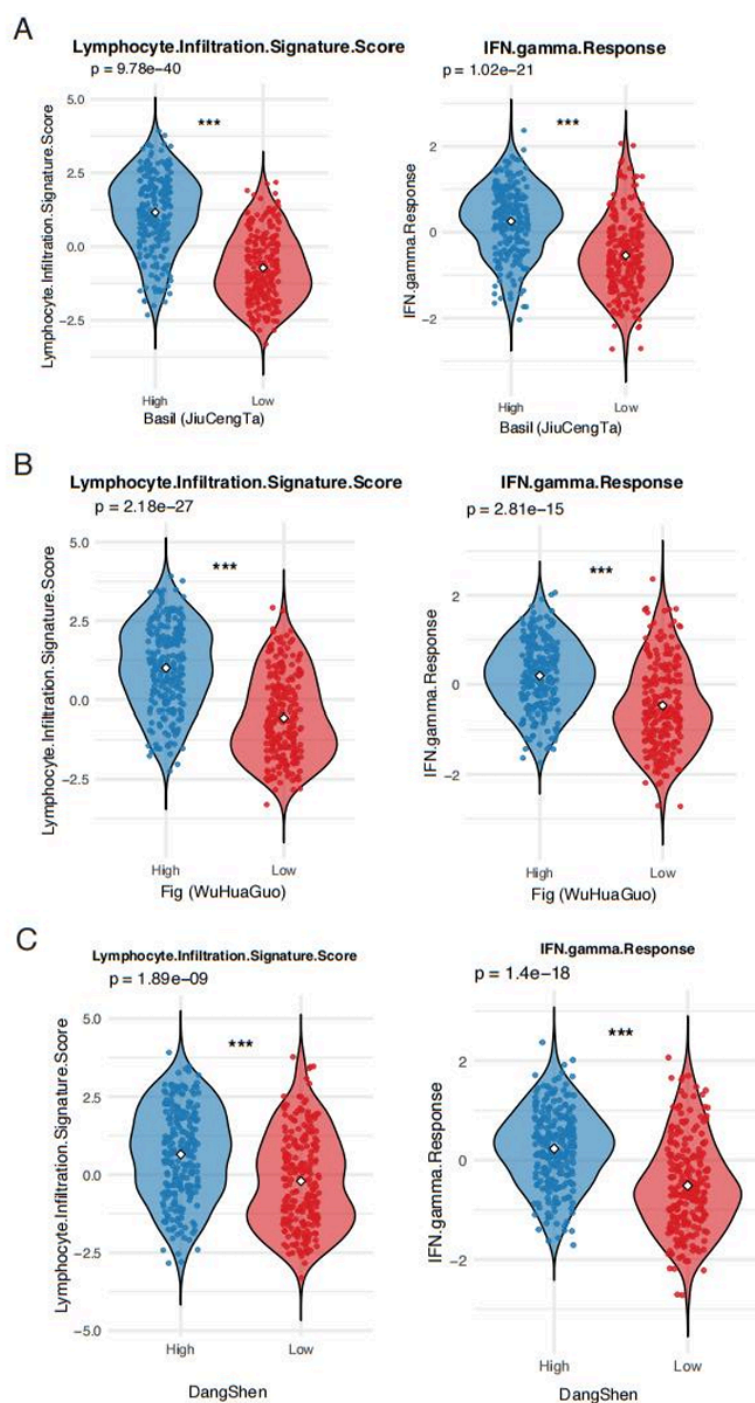


Figure 2. Elevated herb regulatory scores correlate with enhanced immune infiltration and IFN- γ response

Box plots illustrate the relationship between high (right) and low (left) regulatory scores of Basil (A), Fig (B), or DangShen (C) and two immune-related signatures: lymphocyte infiltration (top row) and IFN- γ response (bottom row). Patients with higher herb regulatory scores exhibited significantly increased TIL signature levels and IFN- γ response scores compared with the low-score group, suggesting a more robust anti-tumor immune microenvironment. Statistical significance was determined using the Wilcoxon rank-sum test, with p-values indicated on each panel.

3.3. Dose-dependent cytotoxic effects of Basil, Fig, and DangShen extracts on B16F10 melanoma cells

To explore the direct anti-tumor efficacy of the three top-ranked herbs, we treated B16F10 melanoma cells with increasing concentrations of Basil (JiuCengTa), Fig (WuHuaGuo), and DangShen (*Codonopsis pilosula*) extracts and measured cell viability via CCK8 assay after 48 hours. As illustrated in Figure 3, all three extracts exhibited dose-dependent cytotoxic effects, albeit with differing potencies. Basil showed the strongest inhibitory activity, achieving an IC₅₀ of 0.1065 mg/mL. By comparison, Fig displayed an intermediate effect (IC₅₀ = 1.189 mg/mL), whereas DangShen required higher concentrations to exert a comparable level of cytotoxicity (IC₅₀ = 6.422 mg/mL). These findings corroborate the earlier survival and immune-infiltration data, indicating that each herb possesses anti-proliferative properties against melanoma cells, with Basil displaying the greatest potency in this in vitro setting.

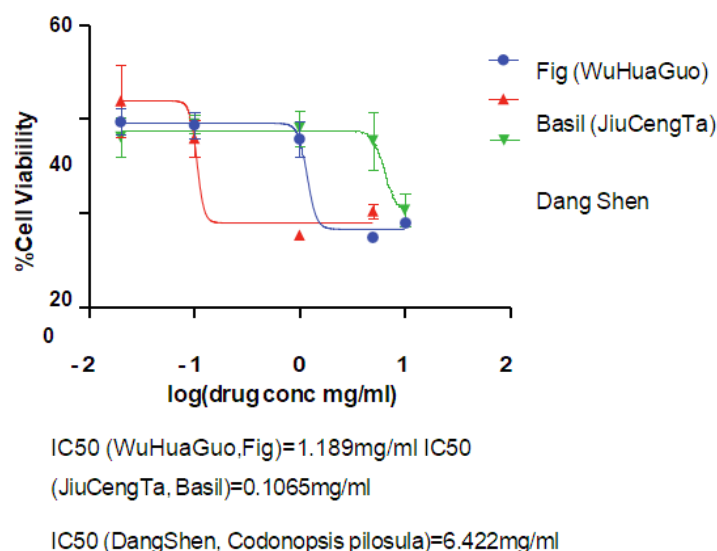


Figure 3. Dose-dependent cytotoxic effects of Basil, Fig, and DangShen extracts on B16F10 cells

A line graph depicts the percentage of viable B16F10 melanoma cells (y-axis) after 48 hours of treatment with serially diluted extracts of Basil (JiuCengTa), Fig (WuHuaGuo), and DangShen (*Codonopsis pilosula*) (x-axis). Cell viability was measured via CCK8 assays. Each herb extract displayed dose-dependent cytotoxicity with distinct IC₅₀ values (noted in the legend), indicating varying potencies in suppressing melanoma cell growth. Error bars (where visible) represent the standard error of the mean from at least three independent experiments.

3.4. Herb-regulated signatures accurately predict GDSC chemosensitivity across multiple models

Wuhuaguo (fig) and Jiucengta (basil)-regulated gene signatures were used to train four classifiers—logistic regression (GLM), random forest (RF), k-nearest neighbors (KNN), and neural network (NNET)—to predict cell-line chemosensitivity (log-IC₅₀) across the GDSC database (Figure 4). For Wuhuaguo, nine compounds (Alisertib, ABT-737, Entinostat, Molibresib, AZ960, Ipatasertib, MK-8776, Vorinostat, Niraparib) achieved robust discrimination (AUC > 0.8) in at least one model (Figure 4A). Notably, the PARP inhibitor Niraparib yielded the highest AUCs—up to ~0.88—under

both RF and NNET, while HDAC inhibitors Entinostat and Vorinostat also surpassed 0.85 with non-linear algorithms (Figure 4A). In contrast, GLMs crossed the 0.8 threshold for only one drug, and KNNs showed variable performance, indicating that RF and NNET better capture the complex, non-linear relationships between herb-regulated gene expression and drug response. When stratified by mechanism, PARP inhibitors exhibited the strongest median predictability ($AUC \approx 0.87$), followed by HDAC inhibitors ($AUC \approx 0.85$), CHK1 inhibitors ($AUC \approx 0.84$), and BET inhibitors ($AUC \approx 0.82$); unclassified agents fell in between (Figure 4B). Regarding Jiucengta, similar results were achieved. Six compounds—Palbociclib, MK-8776, 2-fluoroAraA (fludarabine), Molibresib, Niraparib, and Vorinostat—surpassed an AUC of 0.8 under at least one algorithm, with Random Forest and Neural Network again delivering the highest AUCs across those drugs (Figure 4C). The drug-class boxplot (Fig. 4D) confirms that PARP inhibitors retain the highest median predictability (~ 0.87), followed by HDAC inhibitors (~ 0.85), BET inhibitors (~ 0.83), and purine analogs (~ 0.82). These results highlight particularly high predictive power for DNA damage–targeting (PARP) and epigenetic (HDAC) therapies using herb-regulated gene panels, and confirm that ensemble and deep-learning approaches (RF, NNET) deliver the most consistent, high-accuracy drug-sensitivity predictions.

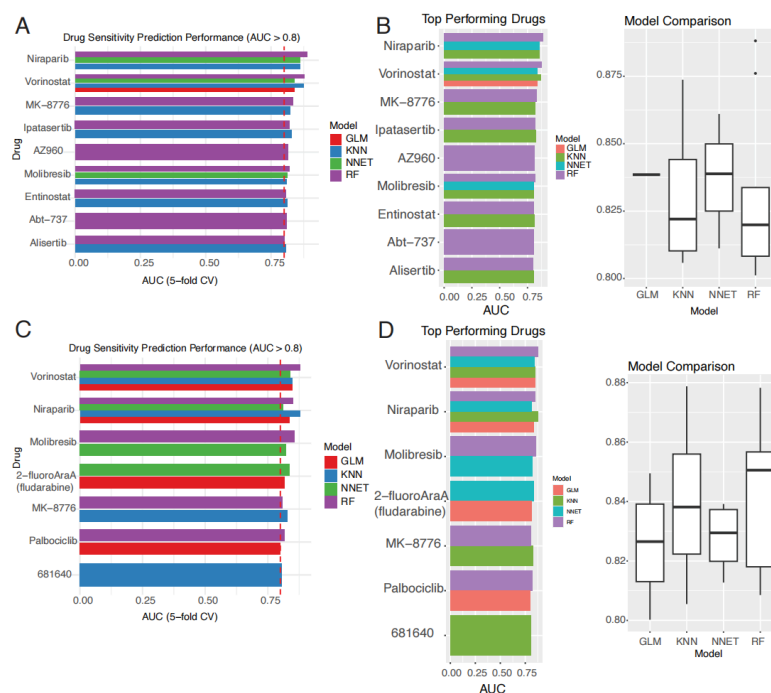


Figure 4. Herb-regulated gene signatures predict chemosensitivity across multiple drug classes using machine and deep learning models

(A) Bar plot showing area under the ROC curve (AUC) values for nine drugs using Wuhuaguo (Fig)-regulated gene signatures across four classifiers: logistic regression (GLM), random forest (RF), k-nearest neighbors (KNN), and neural network (NNET). (B) Left: Top-performing drugs ($AUC > 0.8$) for Fig signature. Right: Boxplot comparing AUC distributions across classifiers, demonstrating superior performance of RF and NNET. (C) Bar plot of AUC values for eight drugs using Jiucengta (Basil)-regulated gene signatures across the same four classifiers. (D) Left: Top-performing drugs for Basil signature. Right: Boxplot comparing model performance, again showing stronger predictive power for non-linear models (RF and NNET). Both herb signatures yielded high predictive accuracy for DNA damage–targeting (PARP) and epigenetic (HDAC) inhibitors.

3.5. Enhancement of T cell activation by Basil, Fig, and DangShen extracts

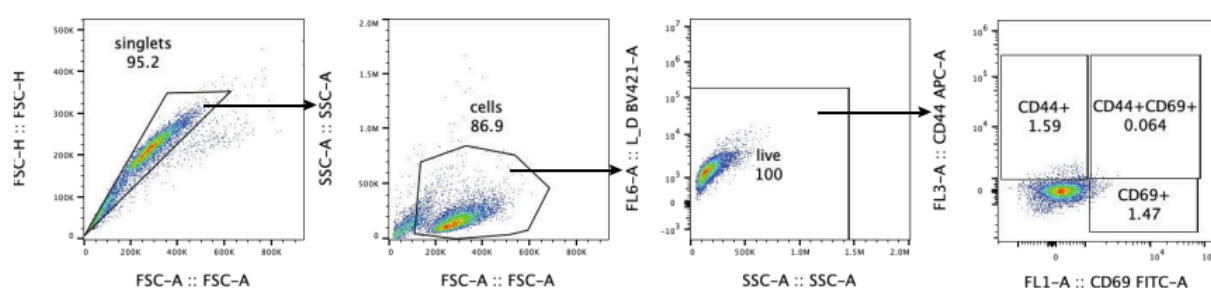
To investigate the immunomodulatory potential of the three top-ranked herbs, we evaluated their effects on T cell activation using mouse peripheral blood mononuclear cells (PBMCs) under anti-CD3/CD28 stimulation. As shown in Figure 5, T cells treated with Basil (JiuCengTa) or Fig (WuHuaGuo) extracts exhibited a clear, dose-dependent increase in activation markers (CD44, CD69) at concentrations of 0.1 mg/mL and above. In contrast, DangShen (*Codonopsis pilosula*) treatment required higher doses (≥ 1 mg/mL) to induce a comparable enhancement in T cell activation, mirroring its relatively weaker cytotoxic effect observed in melanoma cells. These results demonstrate that, while all three herbal extracts support T cell activation, Basil and Fig may confer particularly strong immunostimulatory activity at lower concentrations, aligning with their lower IC50 values in cytotoxic assays.

Across this work, we integrated computational approaches with in vitro validation to elucidate the anti-tumor and immunomodulatory properties of specific TCM herbs in metastatic melanoma. Our analyses of large-scale TCGA data identified Basil, Fig, and DangShen as top candidates linked to improved survival outcomes, heightened lymphocyte infiltration, and augmented IFN- γ response signatures in patients.

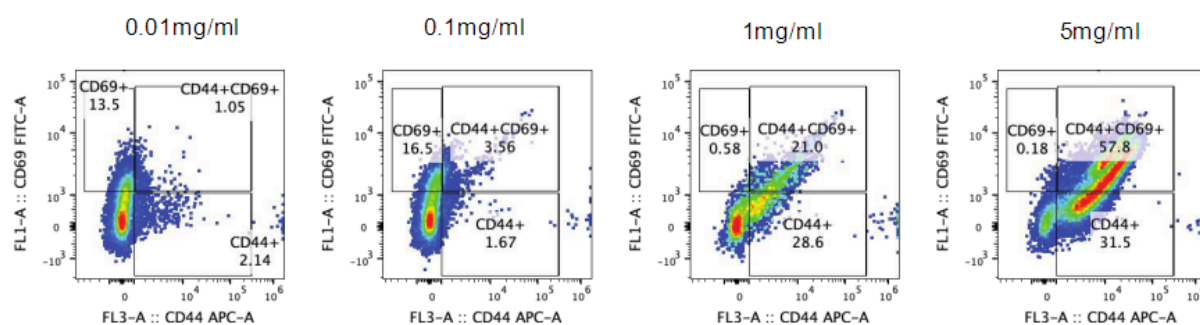
Subsequent in vitro experiments showed that these herbs exert direct growth-inhibitory effects on melanoma cells, with Basil displaying the strongest potency. Furthermore, T cell activation assays revealed that each extract can promote immunostimulatory phenotypes, potentially enhancing anti-tumor immune responses.

By bridging network pharmacology with high-throughput transcriptomic data and functional assays, this study highlights the potential of select TCM herbs as adjunct therapeutic agents in metastatic melanoma. Such evidence supports deeper investigations into the molecular mechanisms by which these herbal compounds may synergize with current immunotherapies, pointing toward innovative, multi-faceted treatment strategies for patients with advanced melanoma.

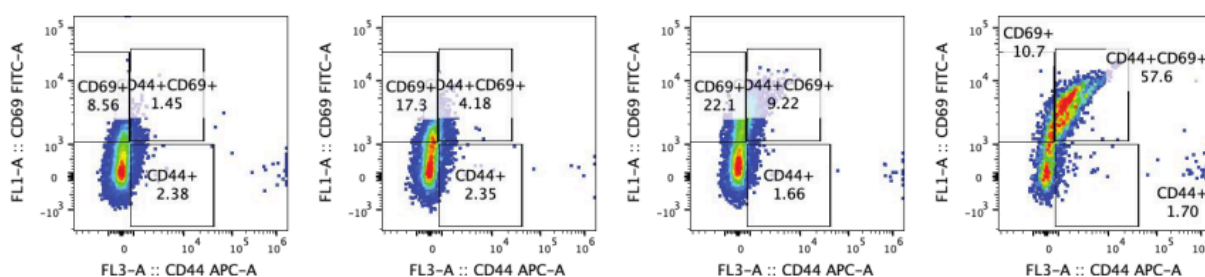
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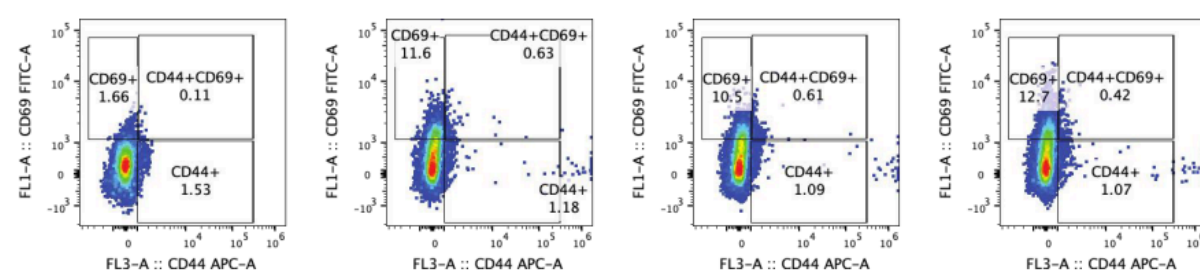


Figure 5. Enhancement of T cell activation by Basil, Fig, and DangShen extracts

Representative flow cytometric data and/or quantitative summaries are shown for T cells stimulated with anti-CD3/CD28 in the presence of increasing concentrations of Basil (JiuCengTa), Fig (WuHuaGuo), or DangShen (*Codonopsis pilosula*) extracts. Panels (A–D) illustrate the dose-dependent upregulation of activation markers (e.g., CD44, CD69) following 48 hours of treatment. Basil and Fig extracts promoted robust T cell activation at lower concentrations, whereas DangShen required higher doses to achieve comparable effects. These findings support the immunostimulatory properties of the three top-ranked herbs, potentially contributing to their observed anti-tumor efficacy.

4. Discussion

This study underscores the growing promise of TCM herbs as adjunct or complementary agents in the management of metastatic melanoma, a disease that continues to challenge current therapeutic

paradigms. By combining computational

analyses of large-scale transcriptomic data with rigorous *in vitro* validation, we provide evidence that specific herbs—namely Fig (WuHuaGuo) and Basil (JiuCengTa) offer measurable anti-tumor benefits. In particular, Basil and Fig demonstrated robust cytotoxic activity against B16F10 melanoma cells at relatively low concentrations and simultaneously enhanced T cell activation, suggesting a dual-pronged mechanism involving both direct tumor cell killing and immune system modulation. Such findings are notable given that immunotherapy has transformed melanoma care by reinvigorating T cell function, yet significant hurdles remain in overcoming primary and acquired resistance to immune checkpoint inhibitors.

One of the central insights to emerge from our computational pipeline was the link between these high-ranking herbs and an immune-active TME. Elevated regulatory scores for Basil, Fig, and DangShen correlated strongly with increased tumor-infiltrating lymphocyte (TIL) signatures as well as higher IFN- γ response levels. TIL enrichment is broadly recognized as a positive prognostic factor in cancer, including melanoma, reflecting enhanced immunosurveillance capable of targeting malignant cells [23,24]. Furthermore, IFN- γ is essential for orchestrating anti-tumor immunity through macrophage activation, amplification of antigen presentation, and promotion of cytotoxic T lymphocyte activity [25]. Our data thus support the notion that certain TCM herbs can modulate critical facets of the TME, helping to shift the balance in favor of an immune response capable of restraining tumor progression.

In addition to these immunological insights, our study expanded into predictive modeling by applying machine learning and deep learning techniques to herb-regulated gene signatures. Using gene expression and drug sensitivity data from the GDSC dataset, we trained four classifiers—logistic regression, random forest (RF), k-nearest neighbors (KNN), and neural networks (NNET)—to assess the ability of herb signatures to predict chemosensitivity. Remarkably, the non-linear models (RF and NNET) consistently outperformed linear models, achieving high predictive accuracy (AUC > 0.8) particularly for DNA damage-targeting (PARP) and epigenetic (HDAC) inhibitors. These results underscore the complex and non-linear nature of herb-drug interactions, which are better captured by ensemble and deep learning methods. By leveraging computational modeling, we not only demonstrated the predictive power of herb-regulated pathways but also highlighted the potential of integrating traditional pharmacological knowledge with state-of-the-art machine learning tools to inform drug repurposing and precision oncology strategies.

Interestingly, while Codonopsis pilosula ranked among the top herbs *in silico* and was linked to improved patient outcomes, its extract required higher doses to achieve comparable cytotoxic and T cell-activating effects *in vitro*. This discrepancy may stem from multiple factors, including the specific molecular constituents of the extract, cell line specificity, or the complex immunoregulatory functions that may manifest more fully *in vivo*. Indeed, many TCM herbs exert their effects through polypharmacological pathways that involve not only direct tumor cell targeting but also interplay with systemic immunity and tumor stroma [26,27]. Consequently, further research using additional melanoma models—or perhaps patient-derived xenografts—may clarify whether Codonopsis pilosula exerts subtler immunomodulatory benefits or synergizes more effectively with other therapeutic agents.

Beyond these immediate observations, our study points to broader opportunities at the interface of network pharmacology and oncology. TCM's multifaceted mechanisms are increasingly amenable to high-throughput computational analyses that map herb-derived compounds to disease-associated gene networks. Systematic approaches of this kind can unearth novel or underexplored herbal candidates, helping to overcome the longstanding challenge of limited, anecdotal data in the

TCM literature. Future work should prioritize isolating and characterizing the specific bioactive constituents responsible for the observed anti-cancer and immunomodulatory activities. Such endeavors would not only facilitate precise mechanistic studies—e.g., investigating the role of pathways like NF- κ B, PI3K/Akt, or MAPK—but also pave the way for rational drug design and improved integration with standard-of-care immunotherapies.

Ultimately, while these findings are promising, the path to clinical translation demands extensive *in vivo* validation and carefully designed clinical trials. Key considerations for the next phase of research include pharmacokinetics, potential off-target effects, and synergistic interactions with ICIs or targeted therapies. Nevertheless, by bridging ancient pharmacopeia with 21st-century computational biology, this study offers a model for how TCM can be systematically probed and innovatively repurposed in the modern oncological landscape. If confirmed in more complex systems, Basil, Fig, DangShen, and other top-ranked herbs could serve as valuable complements in combination regimens, bolstering both the direct tumor-killing capacity and immunotherapeutic efficacy against metastatic melanoma.

5. Conclusion

In conclusion, our integrative approach—combining large-scale transcriptomic analyses with *in vitro* functional assays—demonstrates that Basil (JiuCengTa) and Fig (WuHuaGuo) exert notable anti-tumor effects in metastatic melanoma, mediated by both direct cytotoxicity and immunostimulatory properties. These findings build upon mounting evidence that natural compounds, especially from TCM, can modulate the tumor microenvironment in ways that favor anti-tumor immunity. By pinpointing herbs that not only inhibit melanoma cell proliferation but also enhance T cell activation, our study opens new avenues for supplementing existing immunotherapeutic strategies, particularly for patients who develop resistance to current regimens. Moreover, through predictive modeling of herb-regulated gene signatures, we show that non-linear machine learning algorithms can accurately forecast chemosensitivity to PARP and HDAC inhibitors, further expanding the translational potential of these herbs as biomarkers or adjuvants. Future research that clarifies the underlying molecular pathways and tests these herbs in more complex *in vivo* models or clinical trials will be instrumental in translating these promising leads into practical therapies. This work illustrates how bridging ancient medicinal wisdom with cutting-edge computational biology can uncover innovative and potentially synergistic approaches for modern cancer treatment.

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