ORIGINAL RESEARCH

Multi-Omics Analysis Reveals the Role of Changes in Platelet Activation Pathways in the Survival Outcome of Patients with Esophageal Squamous Cell Carcinoma

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ABSTRACT

Objective • The objective of this study was to integrate metabolomics and transcriptomics data to identify key diagnostic and prognostic markers for esophageal squamous cell carcinoma (ESCC). Plasma samples were collected from 85 ESCC patients at different stages and 50 healthy volunteers for non-targeted metabolomic analysis.

Methods • Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was employed for non-targeted metabolomic analysis. Subsequently, we integrated the metabolomic data with transcriptomic data from the Gene Expression Omnibus (GEO) and prognosis data from The Cancer Genome Atlas Program (TCGA) to perform pathway analysis. Our focus was on pathways that involve both metabolites and upstream genes, as they often exhibit higher accuracy.

Results • Through the integration of metabolomics and transcriptomics, we identified significant alterations in the platelet activation pathway in ESCC. This pathway involves the participation of both metabolites and genes, making it a more accurate reflection of pathological changes associated with the disease. Notably, metabolite arachidonic acid (AA) and chemokine receptor type 2(*CXCR2*) were significantly downregulated in ESCC, while genes collagen type I alpha 1(*COL1A1*), collagen type I alpha 2(*COL1A2*), collagen type III alpha 1(*COL3A1*), type 3 inositol 1,4,5-trisphosphate receptor (*ITPR3*), and insulin-like growth factor II mRNA binding protein 3(*IGF2BP3*) were significantly upregulated, indicating the presence of tumor-induced platelet activation in ESCC.

Further analysis of prognosis data revealed that high expression of *COL1A1, IGF2BP3*, and *ITPR3* was associated with a favorable prognosis for ESCC, while high *CXCR2* expression was linked to an adverse prognosis. In addition, we combined *COL1A1, ITPR3, IGF2BP3, CXCR2*, and AA to form a diagnostic biomarker panel. The receiver operating characteristic curve (ROC) demonstrated excellent diagnostic capability (AUC=0.987).

Conclusion • Our study underscores the significant role of platelet activation pathways and related genes in the diagnosis and prognosis of ESCC patients. These findings offer promising insights for improving the clinical management of ESCC. (*Altern Ther Health Med.* 2024;30(10):538-544).

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INTRODUCTION

Esophageal cancer ranks as the sixth leading cause of cancer-related mortality, accounting for over 400 000 annual deaths.[1] This cancer encompasses two subtypes, esophageal adenocarcinoma (EA) and ESCC, with approximately 90% of cases classified as ESCC.[2] The elusive etiology of ESCC has left the clinical community devoid of effective therapeutic targets, thereby contributing to the persistently low five-year survival rates among ESCC patients.[3]

Metabolomics, a discipline investigating the systematic changes in small molecular compounds within an organism under the influence of diseases and other stimuli,[4] has gained widespread application in the study of various malignancies, including ESCC.[5–7] Metabolites, as the most downstream and ultimate representation of the cellular phenotype in the context of systems biology, serve not only as essential nutrients

Figure 1. The diagram of data generation and study design. Metabolomic analysis and transcriptomic analysis serve as the initial investigative phases (A); the focus lies in the integration of metabolomic and transcriptomic data to pinpoint pathways involving the concurrent participation of metabolites and genes (B). Subsequently, the identified metabolic pathways are subjected to prognostic analysis in another validation cohort (C), thereby enabling the selection of potential targets within ESCC.

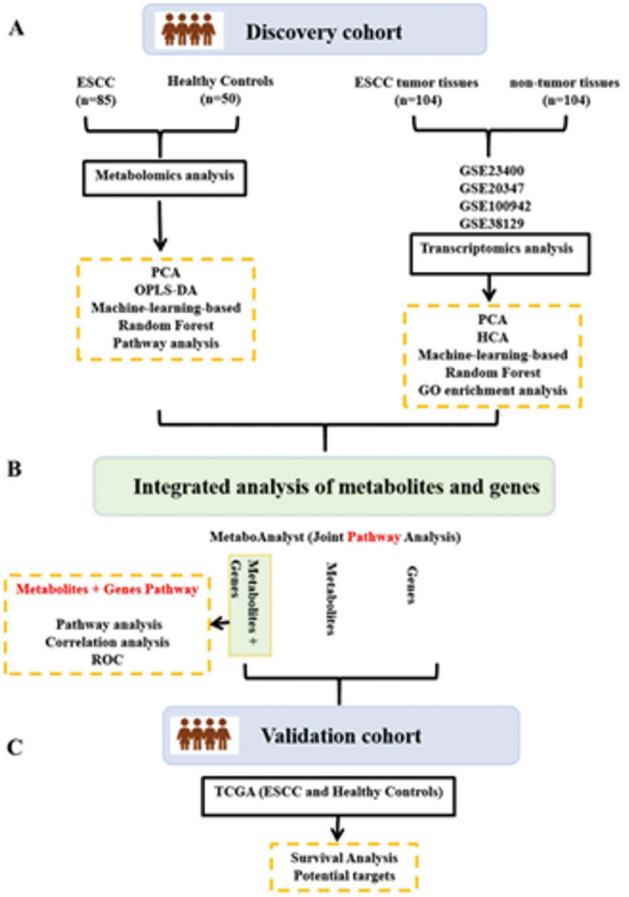


Table 1. Clinical pathologic characteristics of samples used in global metabolomics of this study.

Characteristics			ESCC patients	Healthy controls
No. of subjects			85	50
Gender	Male		62	33
	Female		23	17
Age	mean		62	61
	range		41-78	42-80
Grade	G1		41	/
	G2		41	/
	G3		3	/
	G4		0	/
TNM Staging	T(Tumor)	T0	4	/
		T1	13	/
		T2	14	/
		T3	38	/
		T4	16	/
	N(Node)	N0	51	/
		N1	20	/
		N2	10	/
		N3	4	/
	M(metastasis)	M0	85	/

but also as critical cellular modulators and signaling molecules that play a pivotal role in initiating and sustaining carcinogenesis. Metabolites may exert effects that are equivalent to, or even surpass, those traditionally attributed to proteins or genes. Over the past decade, the increasing recognition of the pivotal role that metabolites can play as crucial biomarkers in cancer has elevated the significance of metabolomics in providing a comprehensive perspective on various aspects of cancer.[8,9] In particular, metabolomics has contributed significantly to characterizing the unique features of cancers, revealing distinct metabolic profiles associated with cancer, and identifying novel members of the cancer genome.[10] Beyond categorizing these cancer "omics," metabolomics aids in a deeper understanding of their underlying molecular mechanisms. Thus, diseases that involve the concurrent regulation of metabolites and genes (or proteins) are better suited to accurately delineate the patterns governing disease onset and progression, offering more precise insights into the mechanisms underlying these diseases.

In this study, we aimed to integrate metabolomics and transcriptomics to identify key diagnostic and prognostic targets for ESCC, with the overarching goal of improving patient survival rates.

MATERIALS AND METHODS

In this study, we employed non-targeted metabolomic analysis based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) to investigate plasma samples from 85 individuals with ESCC and 50 healthy control subjects. The differentially regulated metabolites obtained from this analysis were integrated with data from the GEO and TCGA databases. The primary objective was to identify dysregulated pathways in ESCC that involve both metabolites and genes, offering insights into the underlying mechanisms of ESCC. The overall conceptual framework of this study is illustrated in Figure 1.

Sample collection and preparation

Plasma samples were collected at the Affiliated Hospital of North Sichuan Medical College from ESCC patients (n=85) and healthy individuals (n=50). Relevant scientific research ethical requirements were followed, and informed consent was obtained from all participants. To ensure the reliability of plasma samples, all samples were taken from individuals with an empty stomach before any treatment was administered. Samples were staged according to the 7th edition of the American Joint Committee on Cancer, and cancers were divided into five stages (0, I, II, III, and IV).

To ensure the reliability of the results, we formulated strict sample exclusion standards. (1) The exclusion criteria for healthy samples were: 1) suffering from any disease; 2) obvious abnormalities in blood test biochemical indicators. (2) The exclusion criteria for ESCC samples were: 1) suffering from other solid tumors; 2) suffering from metabolic diseases, such as diabetes, hypertension, or hyperlipidemia; 3) suffering from a chronic disease, such as cardiovascular disease or emphysema. Detailed sample information is summarized in Table 1.

Plasma samples were pre-treated by acetonitrile precipitation of protein and were then subjected to LC-ESI-MS. The specific steps were as follows. Frozen plasma samples were thawed in a refrigerator at 4°C, and 450 ul cold acetonitrile was added to 150 ul plasma. After vortex mixing, the sample was placed at 4°C for 10 min and then centrifuged at 12 000 r/min for 10 minutes at 4°C. The supernatant was transferred to a fresh tube and dried under a stream of nitrogen, then redissolved in 100 ul of acetonitrile/water solvent (3:1, v/v). The supernatant was centrifuged at 10 000 r/min for 10 minutes at 4°C prior to LC-MS.

Metabolomics data analysis process

LC-MS method for metabolomics data collection. Mass spectrometry was performed on a Japan Shimadzu UHPLC LC-30A system coupled to a TripleTOF 4600™ mass spectrometer with an electrospray ionization (ESI) probe, controlled by AnalystTF 1.7.1 software (Applied Biosystems/MDS SCIEX Corporation). Chromatographic separation was performed on a Waters ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 ×100 mm). The column temperature was 35°C, and the flow rate was 0.25 mL/min. Mobile phase A was 0.1% formic acid in water, and mobile phase B was acetonitrile. An injection volume of 5 μL was used for both positive and negative electrospray ionization (ESI) modes. The positive and negative ESI modes were adopted. The full scan was used as the scanning mode, ranging from m/z 50–1000, and nitrogen was used for all gas paths, using the following operation parameters. Ionspray Voltage (kV) 5.0/ -4.5; Curtain Gas(CUR) 30 psi; Collision Gas(CAD) High; Temperature (°C) 500; Turbo Gas (arb) 40/50; Nebulizer Gas 2 (arb) 45/50; Declustering Potential (V) 50 / -50; Focusing Potential (V) 15/-15.

Statistical analysis methods for metabolomics data. Raw LC-MS data files were initially converted to mzXML format using Wiff to mzData translator software (version 1.0.0.4, Applied Biosystems/MDS Sciex). Nonlinear retention time alignment, peak discrimination, filtering, alignment, matching, and identification were subsequently conducted using open-source software XCMS. The structures of dysregulated metabolites were identified in the following steps. First, free databases, such as HMDB, Massbank, and METLIN, were searched with exact molecular weights. Second, high-resolution LC-MS/MS spectra were used for further identification, applying standard compounds to verify the potential structures. Finally, the identification score was obtained by the scoring metric. Following data preprocessing, multivariate statistical data analysis (MVDA) was employed to select crucial differential metabolites and metabolic pathways.

Analysis of the GEO database.

The Gene Expression Omnibus (GEO) database and The Cancer Genome Atlas (TCGA) database, which encompass tumor-related transcriptomic and clinical information, were utilized in this study. We gathered the transcriptional data and clinical information from these databases, respectively. The GEO2R online analysis tool was used to analyze and extract DEGs. The screening criteria were $P < .05$ and $|\log(FC)| > 2$. Complete characterization analysis of heat map and volcano plot were carried out using an online website, Sangerbox 3.0.[11]

Integrated analysis of metabolomics and transcriptomics data

In this study, we employed the "Joint Pathway Analysis module" of MetaboAnalyst 5.0. This specific module is designed to facilitate the integrated analysis of transcriptomics and metabolomics data at the pathway level. In this context, we directed our focus towards pathways in diseases where both metabolites and genes (or proteins) are concurrently involved in the regulation. We recognized the importance of such pathways in accurately reflecting the patterns of disease onset and progression, thereby providing more precise insights into the mechanisms underlying these diseases. Within this framework, our attention was drawn to the significant alterations observed in the platelet activation pathway among ESCC patients. This approach allowed us further to identify potential targets in the context of ESCC development.

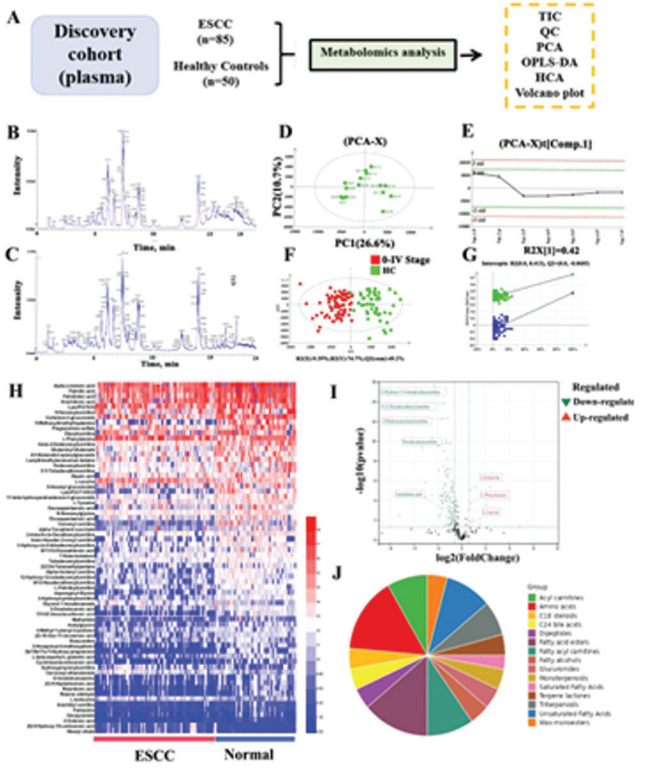
Survival and Diagnostic Analysis of ESCC

To further understand the potential role of platelet activation pathway-related genes in the development of ESCC, we conducted a survival analysis. We obtained survival information for ESCC patients within a 5-year timeframe from the TCGA public database. We employed the R software package "survival" to conduct survival analysis. We employed the online Kaplan-Meier survival curve plotting tool, SangerBox 3.0, to construct risk score models. These models were based on optimal cutoff values, which are values selected to group the data when the Risk column is treated as a continuous numerical variable. The selection of these values relies on the maximization of statistical metrics provided by the R software package maxstat. Subsequently, we categorized the samples into high-expression and low-expression groups to assess the relationship between individual genes within the platelet activation pathway and patient survival. To assess the potential utility of these biomarkers for predicting ESCC, we conducted univariate ROC curve analysis. ROC curves provide information regarding the sensitivity and specificity of potential biomarkers. Given that ESCC is a complex ailment involving systemic perturbations of biochemical pathways, a biomarker panel containing multiple markers rather than a single one offers superior discriminatory capabilities and pathophysiological insights. Subsequently, we carried out correlation analyses for the genes and metabolites associated with this pathway using an online website, Sangerbox 3.0.

Statistic analysis

In metabolomics data analysis, R statistical software (Version 3.6.0), was used for peak extraction, alignment, and filling. The loaded packages included xcms, MSnbase, CAMERA, stringr, etc. The obtained two-dimensional data matrix was first analyzed with Z-scores to identify statistical Outliers. Depending on whether the data was normally

Figure 2. Metabolomics data analysis. The workflow diagram for metabolomics analysis (A). Representative TICs were generated through LC-(±)ESI-MS for the samples (B, C). PCA of the QC samples (D, E). Score plots from OPLS-DA models (F). Validation plots from partial least squares discriminant analysis (PLS-DA) models (G), utilizing LC-(+) ESI-MS data. HCA illustrating the perturbed metabolites (H). Volcano plot representation of identified metabolites (I). Metabolic pathway enrichment analysis for metabolites exhibiting significant differences (J).



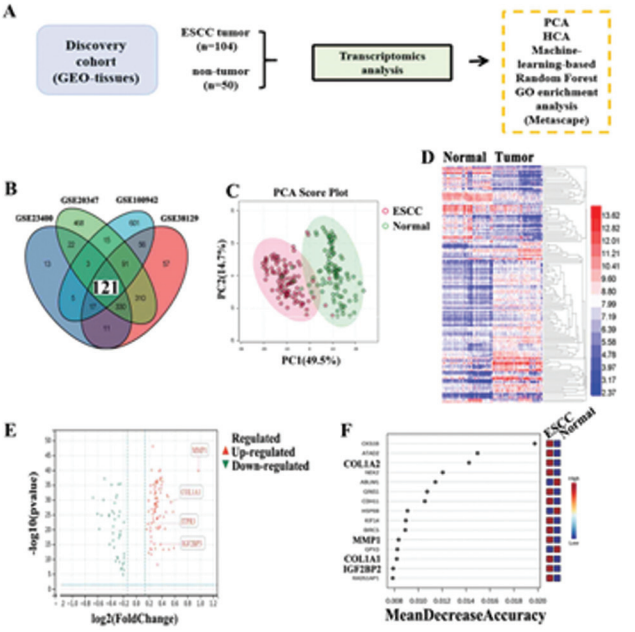
distributed or not, paired *t* tests or Wilcoxon-paired tests were used. Considering the 5% error detection rate, the *P* value of multiple comparisons was adjusted according to the Benjamin-Hochberg method. The significant alpha level was set to $P < .05$, and all results are expressed as mean ± Standard error (SEM) of the mean. GraphPad Prism 8.0 and IBM SPSS 16 statistical software for statistical analysis were also utilized.

RESULTS

Metabolomics data analysis

A stable analytical method is important to obtain reliable data from metabolomics analysis. The workflow for metabolomic analysis is depicted in Figure 2A. Typical total ion chromatograms (TICs) indicating good chromatographic separation are shown in Figure 2B, C. Unsupervised principal component analysis (PCA) was performed on all QC samples (Figure 2D, E). These results indicated that chromatographic separation and mass measurement were highly stable and reproducible throughout the sequence. The LC-ESI-MS data sets obtained in both positive and negative ion modes contained 2468 and 1576 peaks, respectively. Based on

Figure 3. Analysis of the transcriptomics data. The workflow diagram for transcriptomics data analysis (A). A Venn diagram depicting the overlap of differentially expressed genes among the four GSE datasets (B). PCA (C) and HCA (D) of the 121 DEGs in both non-tumor and ESCC tumor tissues. A volcano plot (E) and a machine-learning-based random forest model (F) for representation.



multivariate statistical data analysis, orthogonal partial least squares discriminant analysis (OPLS-DA) in SIMCA-P (Umetrics AB, Umeå, Sweden) and permutation tests with 100 iterations were performed (Figure 2F, G) to obtain an overview of the plasma LC-MS data from ESCC patients and healthy controls (HC). Hierarchical clustering analysis (HCA) was employed to assess the dysregulation of these metabolites, and the results are presented in Figure 2H. The overall distribution of these dysregulated metabolites is visually depicted in the volcanic map (Figure 2I), particularly with significant changes in lipid and amino acid metabolites. Subsequently, a total of 277 metabolites were successfully identified in the context of ESCC compared to the normal control group. These metabolites are categorized as illustrated in Figure 2J.

Analysis of the GEO database

We analyzed four datasets: GSE23400, GSE20347, GSE100942, and GSE38129. The overall analysis workflow is illustrated in Figure 3A, with specific steps as follows: (1) The penny analysis filtered out 121 Differentially Expressed Genes (DEGs), comprising 80 upregulated genes (66.1%) and 41 down-regulated genes (33.9%) (Figure 3B). PCA and HCA of these 121 DEGs from both non-tumor and ESCC tumor tissues are depicted in Figure 3C and D. (2) A machine-learning-based random forest model and volcano plots uncovered critical gene molecules, especially the *COL1A1, COL1A2, IGF2BP3* and other genes related to platelet activation pathway (Figure 3E, F).

Figure 4. Integrated analysis of metabolites and key genes. The workflow diagram for the integrated analysis (A). Analysis of metabolic pathways that involve both metabolites and genes (B). The trend of molecular changes in the platelet activation pathway enrichment is depicted in (C). A correlation coefficient diagram illustrating potential diagnostic biomarkers related to the platelet activation pathway in ESCC (D).

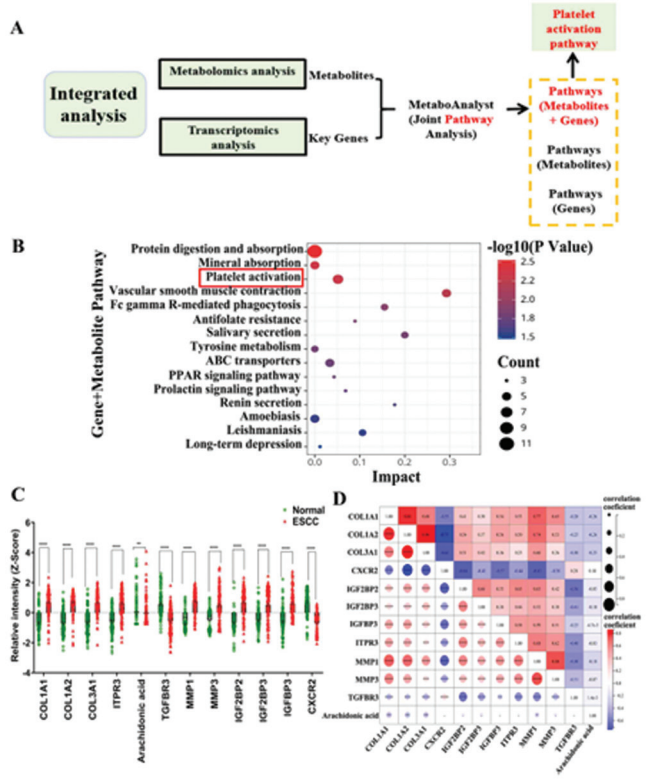
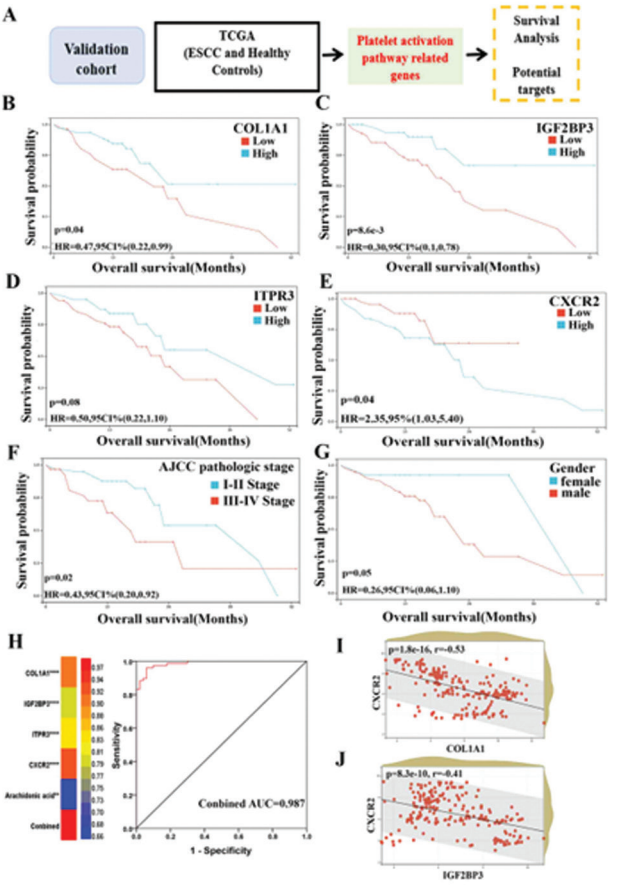


Figure 5. Survival and diagnostic analysis of ESCC. Validation analysis flowchart (A). Survival analysis for ESCC with respect to *COL1A1* (B), *IGF2BP3* (C), *ITPR3* (D), *CXCR2* (E), AJCC pathologic stage (F), and gender (G). The discriminatory power of the individual and combined potential diagnostic biomarkers is further visualized in (H). A correlation coefficient diagram illustrating these potential diagnostic biomarkers for ESCC (I, J).



Integrated analysis of metabolites and key genes

The integration analysis process of metabolites and key genes is shown in Figure 4A. We observed pathways (Figure 4B) that encompass both metabolites and differential genes, notably showing andindicating significant changes in protein intestinal absorption and degradation, mineral absorption, and platelet activation pathways. Since the differential genes were primarily sourced from ESCC tumor tissues, the enrichment of these differential genes in the protein intestinal absorption and degradation and mineral absorption pathways may not be precise. Hence, we elected to proceed with further investigation into the platelet activation pathway. Molecular changes related to this pathway are depicted in Figure 4C, with most molecules exhibiting substantial upregulation in ESCC (Such as *COL1A1*, *COL1A2*, *COL3A1*, *ITPR3*, *MMP1*, *MMP3*, *IGF2BP2*, *IGF2BP3*, *IGFBP3*). We subsequently constructed correlation coefficient diagrams for these potential diagnostic biomarkers in ESCC (Figure 4D), revealing closer correlations among specific molecules (*COL1A1, IGF2BP3, ITPR3, CXCR2*, AA).

Survival and Diagnostic Analysis of ESCC

The survival and diagnostic analysis process is shown in Figure 5A. Our analysis revealed a favorable correlation between high expression levels of *COL1A1 (P = .04), IGF2BP3 (P = 8.6e-3)*, and *ITPR3(P = .08)* with ESCC prognosis (Figure 5B-D), while high *CXCR2(P = .04)* expression was associated with poorer prognosis (Figure 5E). Furthermore, different AJCC pathologic stages*(P = .02)* (Figure 5F) and gender*(P = .05)* (Figure 5G) had significant impacts on ESCC prognosis. The molecules were arranged in heat maps based on the area under the ROC curve (AUC) values, illustrating their discriminative power (Figure 5H). The outcomes demonstrated that these molecular panels, for diagnosing ESCC patients versus controls, achieved an AUC of 0.987 at the optimal cutoff points (Figure 5H). These findings underscore the reliability of the biomarker panel for distinguishing between ESCC patients and healthy controls. Correlation analysis found that there was a significant negative correlation between *COL1A1(P = 1.8e-16)* and *IGF2BP3 (P = 8.3e-10),* and *CXCR2* (Figure 5I, J).

DISCUSSION

We found that in addition to significant changes in the genes *COL1A1, COL1A2, COL3A1, ITPR3*, and AA involved in the platelet activation pathway, we also found significant changes in pathway-related genes *IGF2BP3, IGFBP3, MMP1, MMP3*, and *CXCR2*. This highlights the comprehensive insights provided by the combination of metabolomics and transcriptomics to gain a more systematic understanding of the functions and regulatory mechanisms of biological molecules, providing new references for further exploration of the mechanisms of ESCC occurrence and development.

The role of platelet activation in ESCC

It is now evident that tumor cells possess the capacity to activate platelets and induce platelet aggregation, a phenomenon known as tumor cell-induced platelet aggregation (TCIPA), and TCIPA plays a pivotal role in tumor growth, angiogenesis, metastasis, and immune evasion.[12] However, activated platelets release *PDGFB,* which helps maintain the vascular barrier within tumors, thereby inhibiting tumor cell dissemination.[13] Our research has shown significant upregulation of *COL1A1*, *COL1A2, COL3A1*, and *ITPR3* genes in the platelet activation pathway. Therefore, we speculate that there is a platelet activation phenomenon in ESCC, which has been confirmed in another ESCC study.[14] Further survival analysis revealed that patients with high *COL1A1* and *ITPR3* expression had significantly better five-year survival rates than those with low expression, suggesting that platelet activation may inhibit ESCC progression. Another ESCC study seems to confirm our findings from another perspective; they found that the mean platelet volume (MPV), which elevation signifies platelet activation, significantly decreased in the plasma of advanced-stage ESCC patients and was strongly associated with poor prognosis.[15] Detailed mechanistic investigations will be necessary to understand ESCC's pathogenesis and provide a foundation for early diagnosis, prognosis, and therapeutic target development.

The role of collagen (Col I), ITPR3, AA, and IGF2BP3 in ESCC

Collagen, a major component of the extracellular matrix, plays a crucial role in platelet activation upon vascular injury.[16] In tumor tissue, type I collagen (Col I) expressed by fibroblasts in the tumor microenvironment (TME) is the main collagen protein in the extracellular matrix (ECM), with two α 1 and 1 α 2 collagen chains, which COL1A1 and COL1A2, respectively16COL1A1 and COL1A2, respectively[17] express express. Our study identified significant upregulation of *COL1A1* and *COL1A2* in ESCC, consistent with prior ESCC research results.[18,19] People generally believe that the accumulation of Col I in the ECM may induce cancer cell proliferation, survival, and migration, often signaling poor diagnosis[20] Nevertheless, our study indicated that high *COL1A1* expression was associated with significantly higher five-year survival rates for patients, suggesting that Col I might inhibit tumor progression by activating platelets within the ESCC tumor tissue or neovasculature. Additionally, recent studies in pancreatic ductal adenocarcinoma (PDAC) found that full-length Col I significantly inhibits tumor development by targeting the DDR1-NRF2 pathway.[21] This implies that Col I may have varying effects in different tumor contexts, and the mechanistic research into its inhibition of ESCC development warrants further exploration.

Our research has revealed significant upregulation of the key gene in the platelet activation pathway, ITPR3, in ESCC patient tissue. Though the survival analysis did not show significant differences, another study on ESCC patients found that high *ITPR3* expression was associated with significantly improved survival rates, suggesting that ITPR3 indeed has an inhibitory effect on ESCC.[22] Activation of ITPR3 on platelets facilitates Ca^{2+} release from the endoplasmic reticulum (ER), further promoting platelet activation.[23] The significant upregulation of *ITPR3* in tumor tissue may cause substantial Ca^{2+} release within tumor cells, which enters the blood vessels and encourages platelet activation, inhibiting ESCC. Moreover, studies have shown that ITPR3 at the mitochondria/ER contact sites can mediate Ca2+ transfer from the ER to the mitochondria, which can induce cell apoptosis when excessive Ca2+ uptake in mitochondria.[24] The impact of ITPR3 on promoting ESCC cell apoptosis through excess Ca^{2+} uptake into mitochondria needs further investigation.

AA can be converted to unstable prostaglandin G2 (*PGG2*) and prostaglandin H2 (*PGH2*) by enzymes like cyclooxygenases (COX), which, in turn, produce thromboxane A2 (TXA2), PGI2, and other molecules, leading to platelet activation.[25] Our research discovered that AA levels in the plasma of ESCC patients had a significant reduction compared with the control group. In contrast, as tumors progress, cancer cells may promote an increase in AA levels by activating enzymes like phospholipase A (PLA).[26] The specific role of AA in ESCC requires further study.

IGF2BP3 is a member of the RNA-binding protein (RBP) family[27] and was significantly overexpressed in ESCC patient tissue in our study. IGF2BP3 not only plays a role in tumor angiogenesis but also exerts its biological functions in cancer through mRNA splicing, stability, and translational regulation.[28] Nevertheless, survival analysis based on ESCC RNA-seq data from the TCGA database showed that patients with high IGF2BP3 expression had significantly better survival rates (Figure 5C). This contradicts the results observed in other studies where high IGF2BP3 expression in ESCC patients undergoing surgery-only treatment was associated with significantly lower survival rates.[29,30] These contrasting results suggest that IGF2BP3 has an inhibitory role at the transcriptional level, and the specific mechanisms need further investigation.

The role of CXCR2 in ESCC.

Considering the relationship between platelet activation and *CXCL8/CXCR2,*[31] we studied *CXCR2* gene expression in ESCC tissue and found that it was significantly downregulated, a result confirmed by another ESCC study.[32] The downregulation

of *CXCR2* in ESCC seems to indicate its role in inhibiting tumor progression. Nonetheless, survival analysis showed that patients with low *CXCR2* expression had significantly higher five-year survival rates than those with high expression (Figure 5E), suggesting that *CXCR2* still has a tumor-promoting role in ESCC. Pearson correlation analysis showed significant negative correlations between *CXCR2* expression and *IGF2BP3* and *COL1A1* (Figure 5I, J), implying that platelet activation may inhibit ESCC development by downregulating *CXCR2* expression. However, further research is necessary to uncover the specific mechanisms.

Limitations

This study has some limitations, including the relatively small number of participants in both the metabolomics investigation and the analysis of public data (GEO and TCGA data mining). We plan to address this limitation by expanding the sample size for further in-depth analysis in subsequent stages.

In summary, through multi-omics data analysis, we identified a close association between the platelet activation pathway and related genes with ESCC. Furthermore, we identified *COL1A1, ITPR3, IGF2BP3, CXCR2*, and AA as a diagnostic biomarker panel for accurate ESCC diagnosis. Additionally, *COL1A1, IGF2BP3*, and *CXCR2* were found to be closely associated with ESCC prognosis. In-depth mechanistic studies targeting the platelet activation pathway and related genes not only contribute to elucidating the development mechanisms of ESCC but also hold the potential to discover more effective therapeutic targets for ESCC, ultimately aiming to improve patient survival rates.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION

Huiqing Wang and Zhenyan Li contributed equally to the work.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424. doi:10.3322/caac.21492
2. Abnet CC, Arnold M, Wei WQ. Epidemiology of Esophageal Squamous Cell Carcinoma. *Gastroenterology*. 2018;154(2):360-373. doi:10.1053/j.gastro.2017.08.023
3. Baba Y, Yoshida N, Kinoshita K, et al. Clinical and Prognostic Features of Patients With Esophageal Cancer and Multiple Primary Cancers: A Retrospective Single-institution Study. *Ann Surg*. 2018;267(3):478-483. doi:10.1097/SLA.0000000000002118
4. Rinschen MM, Ivanisevic J, Giera M, Siuzdak G. Identification of bioactive metabolites using activity metabolomics. *Nat Rev Mol Cell Biol*. 2019;20(6):353-367. doi:10.1038/s41580-019-0108-4
5. Yu M, Wen W, Yi X, Zhu W, Aa J, Wang G. Plasma Metabolomics Reveals Diagnostic Biomarkers and Risk Factors for Esophageal Squamous Cell Carcinoma. *Front Oncol*. 2022;12:829350. doi:10.3389/fonc.2022.829350
6. Zhao J, Zhao X, Yu J, et al. A multi-platform metabolomics reveals possible biomarkers for the early-stage esophageal squamous cell carcinoma. *Anal Chim Acta*. 2022;1220:340038. doi:10.1016/j.aca.2022.340038
7. Chuchueva N, Carta F, Nguyen HN, et al. Metabolomics of head and neck cancer in biofluids: an integrative systematic review. *Metabolomics*. 2023;19(9):77. doi:10.1007/s11306-023-02038-2
8. Wishart D. Metabolomics and the Multi-Omics View of Cancer. *Metabolites*. 2022;12(2):154. doi:10.3390/metabo12020154
9. Montaner J, Ramiro L, Simats A, et al. Multilevel omics for the discovery of biomarkers and therapeutic targets for stroke. *Nat Rev Neurol*. 2020;16(5):247-264. doi:10.1038/s41582-020-0350-6
10. Sun C, Wang A, Zhou Y, et al. Spatially resolved multi-omics highlights cell-specific metabolic remodeling and interactions in gastric cancer. *Nat Commun*. 2023;14(1):2692. doi:10.1038/s41467-023-38360-5
11. Shen W, Song Z, Zhong X, et al. Sangerbox: A comprehensive, interaction-friendly clinical bioinformatics analysis platform. *iMeta*. 2022;1(3):e36. doi:10.1002/imt2.36
12. Haemmerle M, Stone RL, Menter DG, Afshar-Kharghan V, Sood AK. The Platelet Lifeline to Cancer: challenges and Opportunities. *Cancer Cell*. 2018;33(6):965-983. doi:10.1016/j.ccell.2018.03.002
13. Zhang Y, Cedervall J, Hamidi A, et al. Platelet-specific PDGFB ablation impairs tumor vessel integrity and promotes metastasis. *Cancer Res*. 2020;80(16):3345-3358. doi:10.1158/0008-5472.CAN-19-3533
14. Wang J, Liu H, Shao N, et al. The clinical significance of preoperative plasma fibrinogen level and platelet count in resectable esophageal squamous cell carcinoma. *World J Surg Oncol*. 2015;13(1):157. doi:10.1186/s12957-015-0543-4
15. Sun SY, Zhao BQ, Wang J, et al. The clinical implications of mean platelet volume and mean platelet volume/platelet count ratio in locally advanced esophageal squamous cell carcinoma. *Dis Esophagus*. 2018;31(2):1-6. doi:10.1093/dote/dox125
16. Nuyttens BP, Thijs T, Deckmyn H, Broos K. *Platelet Adhesion to Collagen*. Vol 127. 2011.
17. Fang S, Dai Y, Mei Y, et al. Clinical significance and biological role of cancer-derived Type I collagen in lung and esophageal cancers. *Thorac Cancer*. 2019;10(2):277-288. doi:10.1111/1759-7714.12947
18. Li J, Wang X, Zheng K, et al. The clinical significance of collagen family gene expression in esophageal squamous cell carcinoma. *PeerJ*. 2019;7(10):e7705. doi:10.7717/peerj.7705
19. Wang Z, Chen M, Qiu Y, et al. Identification of potential biomarkers associated with immune infiltration in the esophageal carcinoma tumor microenvironment. *Biosci Rep*. 2021;41(2):BSR20202439. doi:10.1042/BSR20202439
20. Li J, Ding Y, Li A. Identification of COL1A1 and COL1A2 as candidate prognostic factors in gastric cancer. *World J Surg Oncol*. 2016;14(1):297. doi:10.1186/s12957-016-1056-5
21. Su H, Yang F, Fu R, et al. Collagenolysis-dependent DDR1 signalling dictates pancreatic cancer outcome. *Nature*. 2022;610(7931):366-372. doi:10.1038/s41586-022-05169-z
22. Parthasarathi KTS, Mandal S, George JP, et al. Aberrations in ion channels interacting with lipid metabolism and epithelial-mesenchymal transition in esophageal squamous cell carcinoma. *Front Mol Biosci*. 2023;10:1201459. doi:10.3389/fmolb.2023.1201459
23. Rosa N, Sneyers F, Parys JB, Bultynck G. Type 3 IP3 receptors: The chameleon in cancer. In: *International Review of Cell and Molecular Biology*. Vol 351. Elsevier Inc.; 2020:101-148. doi:10.1016/bs.ircmb.2020.02.003
24. Szalai G, Krishnamurthy R, Hajnóczky G. Apoptosis driven by IP(3)-linked mitochondrial calcium signals. *EMBO J*. 1999;18(22):6349-6361. doi:10.1093/emboj/18.22.6349
25. Banerjee D, Mazumder S, Kumar Sinha A. *Involvement of Nitric Oxide on Calcium Mobilization and Arachidonic Acid Pathway Activation during Platelet Aggregation with Different Aggregating Agonists*. Vol 12.; 2016. www.ijbs.org. doi:10.59566/IJBS.2016.12025
26. Koundouros N, Karali E, Tripp A, et al. Metabolic Fingerprinting Links Oncogenic PIK3CA with Enhanced Arachidonic Acid-Derived Eicosanoids. *Cell*. 2020;181(7):1596-1611.e27. doi:10.1016/j.cell.2020.05.053
27. Marquardt KPKH, Sirbasku DA. *Human Platelet-Derived Mitogens. I. Identification of Insulinlike Growth Factors I and II by Purification and N#{176} Amino Acid Sequence Analysis*. Vol 74.; 1989.
28. Yang Z, Wang T, Wu D, Min Z, Tan J, Yu B. RNA N6-methyladenosine reader IGF2BP3 regulates cell cycle and angiogenesis in colon cancer. *J Exp Clin Cancer Res*. 2020;39(1):203. doi:10.1186/s13046-020-01714-8
29. Wakita A, Motoyama S, Sato Y, et al. IGF2BP3 Expression Correlates With Poor Prognosis in Esophageal Squamous Cell Carcinoma. *J Surg Res*. 2021;259:137-144. doi:10.1016/j.jss.2020.10.024
30. Zhao H, Xu Y, Xie Y, et al. m6A Regulators Is Differently Expressed and Correlated With Immune Response of Esophageal Cancer. *Front Cell Dev Biol*. 2021;9:650023. doi:10.3389/fcell.2021.650023
31. Goto M, Liu M. Chemokines and their receptors as biomarkers in esophageal cancer. *Esophagus*. 2020;17(2):113-121. doi:10.1007/s10388-019-00706-8
32. Chen FF, Zhang SR, Peng H, Chen YZ, Cui XB. Integrative genomics analysis of hub genes and their relationship with prognosis and signaling pathways in esophageal squamous cell carcinoma. *Mol Med Rep*. 2019;20(4):3649-3660. doi:10.3892/mmr.2019.10608