

Age-related Immune Infiltration Characteristics of Oral Cancer Based on Public Databases and Their Potential Impact on the Prognosis of Submental Island Flap Reconstruction

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ABSTRACT

This study examined age-related immune infiltration and repair-associated molecular abnormalities in oral cancer using public datasets and explored their potential relevance to submental island flap (SIF) reconstruction.

GEO datasets GSE30784, GSE41613, GSE25099, and GSE37991 were analyzed. Repair-associated candidate genes were selected from literature on flap survival, ischemia–reperfusion injury, wound healing, angiogenesis, inflammation, and matrix remodeling. Differential expression was assessed in GSE30784, followed by screening incorporating age-related trends and prognostic information in GSE41613. Because ages were reported as intervals, findings were interpreted as older-age-related trends rather than results from a strictly defined elderly cohort. External and paired validation used GSE25099 and GSE37991, respectively. Associations with immune infiltration were also examined.

Five key genes—ANGPT2, HIF1A, VEGFC, TGFB1, and MMP9—were identified; IL1B and IL6 were retained for supplementary analysis. All five genes were upregulated in tumor tissues, with consistent findings across validation datasets. In GSE41613, the genes were associated with prognosis, and a higher combined expression score corresponded to poorer overall survival. ANGPT2, HIF1A, VEGFC, and TGFB1 generally correlated positively with myeloid inflammatory features, whereas MMP9 showed a stronger association with M2 macrophage infiltration.

These findings suggest repair-associated molecular and immune abnormalities potentially relevant to SIF reconstruction. The five genes warrant investigation as candidate markers of the local repair microenvironment; their relevance to flap outcomes requires direct clinical validation.

Keywords: Age-related trends; Bioinformatics; Immune infiltration; Oral cancer; Prognosis; Submental island flap

INTRODUCTION

Oral cancer is 1 of the most common malignancies of the head and neck and is associated with a substantial disease burden and marked functional impairment. According to the latest global cancer statistics, cancers

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of the lip and oral cavity still account for considerable morbidity and mortality worldwide, and with ongoing population aging, cumulative exposure to risk factors, and insufficient control in some regions, the disease burden may continue to rise in the future.¹ From the pathological perspective, oral squamous cell carcinoma (OSCC) constitutes the vast majority of oral malignancies and is the central focus of research and clinical treatment in this field.² OSCC not only threatens survival directly but also frequently involves key anatomical sites such as the tongue, floor of mouth, buccal mucosa, gingiva, and hard palate, thereby causing significant impairment in mastication, swallowing, speech, appearance, and social functioning. Therefore, studies on early screening, risk stratification, precision treatment, and postoperative reconstruction for oral cancer are of clear clinical and practical importance.

As population aging continues to intensify, elderly patients account for an increasing proportion of oral cancer cases in clinical practice. Compared with younger patients, elderly patients often present with reduced cardiopulmonary function, poor nutritional status, multiple comorbidities, limited tolerance to radiotherapy and chemotherapy, and increased perioperative risk, making treatment decision-making more complex and the balance between radical tumor resection and functional preservation more difficult to achieve.³ Previous studies have suggested that long-term outcomes in elderly patients with oral cancer are not determined solely by age itself but are jointly influenced by tumor stage, general condition, treatment intensity, accessibility of adjuvant therapy, and postoperative recovery capacity.³ This indicates that, in elderly patients, reliance on conventional TNM staging alone remains insufficient for treatment decision-making. Exploring biological indicators that reflect host status, the local microenvironment, and repair potential is therefore particularly important for optimizing risk stratification and perioperative management.

In recent years, aging has no longer been viewed as a simple accumulation of time but rather as a process accompanied by systemic changes such as immunosenescence and inflammaging. Immunosenescence may manifest as reduced antigen recognition and effector responses, impaired T-cell function, weakened immune surveillance, and persistent low-grade chronic inflammation. Inflammaging, in turn, may promote tumor development, immune escape, and variability in treatment response through cytokine

imbalance, myeloid-cell remodeling, and chronic inflammatory activation.⁴ This issue is particularly important in oral cancer. The oral mucosa is chronically exposed to tobacco, alcohol, betel nut, chronic mechanical irritation, microbial imbalance, and local inflammatory environments, while tumor progression itself induces complex immune remodeling. As a result, the tumor immune microenvironment in elderly patients may be more likely than that in younger patients to display a composite state characterized by insufficient immune efficacy, persistent inflammation, and limited repair capacity.⁴ Such host-background changes driven by aging provide an important basis for understanding the biological differences and postoperative recovery disparities observed in elderly oral cancer.

Oral cancer is not a disease composed solely of tumor cells. The tumor immune microenvironment (TIME) is now recognized as 1 of the core factors determining tumor progression, metastasis, treatment sensitivity, and prognosis. The OSCC microenvironment involves complex interactions among tumor-associated macrophages, regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), dendritic cells, natural killer (NK) cells, and various cytokines, chemokines, and matrix components.⁵ These elements do not function in isolation; rather, under the combined influence of hypoxia, metabolic reprogramming, extracellular matrix remodeling, and abnormal angiogenesis, they form a specific immunosuppressive niche that in turn affects tumor invasion, local recurrence, and patient survival.⁵ Therefore, understanding elderly oral cancer from the perspective of immune infiltration not only helps explain its biological heterogeneity but also offers a new entry point for clarifying the local repair environment after surgery. Particularly in patients undergoing local reconstruction, the immune status within and around the tumor may simultaneously affect post-resection inflammatory control, re-establishment of blood supply, wound healing, and the survival potential of the flap.

In the comprehensive treatment of oral cancer, reconstruction of postoperative defects is crucial for restoring swallowing, articulation, tongue mobility, support of the floor of mouth, and quality of life. For certain small- and medium-sized oral defects, the submental island flap (SIF) has been widely used in oral cancer reconstruction in recent years because of its convenient harvest, relatively reliable blood supply, thin and pliable tissue, favorable color and texture match,

concealed donor site, and relatively low cost.⁶ Previous reports have shown that the SIF achieves a good survival rate and acceptable complication profile in oral soft-tissue reconstruction after oral cancer resection, especially in patients who are unsuitable for complex free-flap reconstruction, require shorter operative time, or have a heavy burden of comorbidities.⁶ These characteristics make the SIF particularly attractive in elderly patients.

Further studies have shown that the SIF is not only suitable for general oral cancer defects but is also feasible in functionally demanding regions such as the tongue. Studies in patients with hemi glossal defects have found that the facial-submental island flap can achieve relatively stable reconstructive outcomes in young, middle-aged, and elderly patients, suggesting a certain degree of applicability across different age groups, particularly in the elderly.⁷ At the same time, studies in patients with oral tongue squamous cell carcinoma have indicated that, under appropriate case selection and standardized neck dissection, the pedicled SIF demonstrates acceptable oncologic safety.⁸ This suggests that the SIF is not merely a technical option but is also closely related to the patient's baseline condition, tumor burden, local blood supply, and microenvironmental status. In other words, factors influencing postoperative flap outcomes are not limited to surgical technique itself; the molecular and immune background of the local tumor region may also be important determinants.

From a biological perspective, however, successful flap reconstruction depends not only on whether transfer has been completed but also on whether a local microenvironment favorable for survival and healing can be established after surgery. Ischemia-reperfusion injury, inflammatory cascades, oxidative stress, apoptosis, microcirculatory reconstruction, neovascularization, and extracellular matrix remodeling are all considered central factors influencing flap survival.^{9,10} A systematic review has shown that, in skin ischemia/reperfusion models related to microsurgical reconstruction, the most frequently reported pathological markers include TNF- α , IL-6, IL-1 β , caspase-3, VEGF, and HIF-1 α , which correspond to inflammation, apoptosis, angiogenesis, and hypoxic response, respectively.⁹ Meanwhile, a systematic review and meta-analysis on gene therapy for improving flap survival indicated that evidence is most concentrated on VEGF and related pathways, while PDGF, FGF, HGF,

and other angiogenesis- and repair-related molecules also show potential value.¹⁰ Together, these findings suggest that flap repair is essentially a dynamic process coupling hypoxia, angiogenesis, inflammation, and matrix remodeling.

When the above repair process is linked to the molecular features of oral cancer itself, important intersections become apparent. First, hypoxia is a fundamental characteristic of the tumor microenvironment. As a key transcription factor in the hypoxic response, HIF-1 α (encoded by the *HIF1A* gene) not only participates in metabolic reprogramming, invasion, and angiogenesis of tumor cells but is also associated with poorer disease-specific survival in OSCC.¹¹ Second, angiopoietin-2 (encoded by the *ANGPT2* gene) is an important regulator of angiogenesis and vascular remodeling, and its high expression in OSCC has been significantly associated with enhanced tumor angiogenesis and reduced overall survival.¹² Third, VEGF-C (encoded by the *VEGFC* gene), a molecule related to lymph angiogenesis and tumor metastasis, has been reported in OSCC to be associated not only with lymph-node metastasis and recurrence but also to be an independent prognostic factor.¹³ These molecules are involved on the one hand, in tumor progression, and on the other hand in adaptation to tissue hypoxia, establishment of microcirculation, and local repair capacity. They therefore have the potential to serve as bridge molecules connecting tumor biology and the outcomes of flap reconstruction.

In addition to hypoxia and angiogenesis, molecules involved in tissue repair and matrix remodeling also warrant attention. TGF- β 1 (encoded by the *TGFB1* gene) is an important signaling molecule in wound healing, inflammatory regulation, fibrosis, and epithelial-mesenchymal transition, and in OSCC it may affect local invasion and recurrence risk. MMP-9 (encoded by the *MMP9* gene) is closely related to basement-membrane degradation, extracellular matrix remodeling, invasion, and metastasis.¹⁴ In wound-healing research, excessively high levels of MMP-9 often indicate an unfavorable healing environment, whereas TGF- β -related signaling is closely associated with the quality of tissue repair.¹⁴ Theoretically, therefore, if tissues surrounding the tumor in elderly patients with oral cancer simultaneously exhibit enhanced hypoxic adaptation, abnormal angiogenesis, TGF- β 1-driven repair imbalance, and excessive matrix degradation mediated by MMP-9, then even after

standardized tumor resection and flap transfer, the postoperative local repair process may still face higher risks. This is precisely why evaluation of SIF suitability solely from an anatomical perspective may be insufficient to explain true differences in reconstructive outcomes.

Meanwhile, with the development of bioinformatics and public-database research, the use of public transcriptomic resources such as GEO and TCGA to conduct molecular typing, prognostic modeling, and immune infiltration analysis in OSCC has become an important direction of research.¹⁵ This shows that public databases can not only help identify stable key molecules but can also provide reproducible and verifiable evidence for clinically relevant questions. However, most existing database-based studies focus on general OSCC prognosis, biomarkers, or tumor immune microenvironment analyses, and few have systematically integrated elderly patients, immune infiltration characteristics, and the prognosis of SIF reconstruction. In particular, when actual flap-related clinical outcomes are difficult to obtain directly from public databases, inferring their potential impact on SIF reconstructive prognosis through repair-related molecular features and immune infiltration characteristics is methodologically reasonable and clinically translatable.

Based on the above background, the present study used public databases to investigate age-related immune infiltration characteristics in oral cancer and integrated immune infiltration analysis with screening of repair-related molecules. The aim was to identify key biological clues that may be associated with the local repair microenvironment and potentially influence the prognosis of SIF reconstruction from the perspectives of hypoxic response, angiogenesis, matrix remodeling, and inflammatory regulation. On the 1 hand, this study may further enrich current understanding of the immune microenvironment and repair-related molecular basis of oral cancer in the context of older-age-related trends. On the other hand, it may provide a new theoretical basis for perioperative evaluation, risk stratification, and individualized management in patients undergoing SIF reconstruction.

MATERIALS AND METHODS

Data Sources

Oral-cancer-related transcriptomic datasets were

retrieved from the Gene Expression Omnibus (GEO) database. GSE30784, GSE41613, GSE25099, and GSE37991 were included in the analysis. GSE30784 was used for differential expression analysis of candidate genes between tumor and normal tissues as well as for immune infiltration analysis; GSE41613 was used for analysis of older-age-related trends and prognosis; GSE25099 was used for external expression validation; and GSE37991 was used for paired validation between tumor tissues and adjacent non-tumor tissues. Because the cases included in the public databases were predominantly OSCC, the analytical object of this study mainly corresponded to OSCC.

Screening of Repair-Related Candidate Genes

Relevant literature on flap survival, ischemia-reperfusion injury, wound healing, angiogenesis, inflammatory response, and matrix remodeling was reviewed to preliminarily compile candidate genes related to tissue repair. These candidates were then evaluated in combination with expression differences in GSE30784, prognostic information in GSE41613, and external validation results in GSE25099 and GSE37991.

Data Preprocessing

Series matrix files and platform annotation files were downloaded for each dataset, and probes were annotated to genes according to the corresponding platform annotation files. The normalization status of each GEO series matrix was checked before downstream analysis. When expression values had already been log2-transformed and normalized by the original data submitters, they were used directly for subsequent analysis. When multiple probes corresponded to the same gene, the probe with relatively stable and representative expression was selected for subsequent analysis. After expression data were organized, sample identifiers and grouping information were unified. Samples in GSE30784 were classified into tumor, normal, and dysplasia groups; GSE25099 into tumor and normal groups; GSE37991 into tumor tissues and adjacent non-tumor tissues according to the pairing relationship; and GSE41613 was used for prognostic analysis based on follow-up information. Because age information in GSE41613 was presented in age intervals, it was treated in this study as a reference for older-age-related trends rather than as a strict age cut-off. Because datasets generated from different platforms were not directly merged for pooled analysis, cross-

dataset batch-effect correction was not performed. Instead, GSE25099 and GSE37991 as well as GSE30784 and GSE41613 were used as independent cohorts to assess the reproducibility and consistency of the expression trends.

Differential Expression Analysis and External Validation

Candidate genes were first analyzed in GSE30784 for expression differences between tumor and normal tissues in order to identify molecules showing stable abnormal expression trends in oral cancer. The results were then externally validated in GSE25099 and further subjected to paired validation in GSE37991 to improve stability and reliability.

Prognostic Analysis

GSE41613 was used as the prognostic cohort. By integrating patient survival status, follow-up time, and the expression of key genes, the relationships between key-gene expression and overall survival were analyzed. Furthermore, a combined expression score based on the 5 key genes was constructed, and patients were divided into a high-score group and a low-score group according to the median. Kaplan-Meier analysis was then used to evaluate survival differences between the 2 groups.

Immune Infiltration Analysis

Based on the expression matrices of GSE30784 and GSE41613, immune infiltration analysis was performed in tumor samples, with a focus on CD8⁺ T cells, Treg cells, M2 macrophages, NK cells, and myeloid inflammatory features. The correlations between the 5 key genes and immune infiltration indicators were further analyzed to assess their relationships with remodeling of the tumor immune microenvironment.

Statistical Analysis

Continuous variables were compared using the Wilcoxon rank-sum test, and paired samples were compared using the paired Wilcoxon test. Survival analysis was performed using the Kaplan-Meier method and univariate Cox regression analysis. Correlations between key genes and immune infiltration indicators were evaluated using Spearman correlation analysis. For differential expression and correlation analyses involving multiple comparisons, *p* values were adjusted using the Benjamini-Hochberg false discovery rate (FDR) method where applicable. A *p* value < 0.05 or an

adjusted FDR value < 0.05 was considered statistically significant, depending on the specific analysis.

RESULTS

Screening Workflow and Results for Repair-Related Key Genes

This study first integrated literature related to flap survival, ischemia-reperfusion injury, wound healing, angiogenesis, inflammatory response, and matrix remodeling to screen candidate genes associated with SIF reconstruction. GEO public datasets related to oral cancer were then incorporated to validate candidate genes by expression analysis, prognostic analysis, external validation, and immune-infiltration-related analysis. The basic information of the included datasets is summarized in Table 1, and the main functions and inclusion rationale of the candidate genes are shown in Table 2. The overall technical workflow of the study is presented in Figure 1. The screening process and results for the 5 key genes are shown in Table 3. Based on prior literature integration, candidate molecules related to hypoxic response, angiogenesis, immune regulation, and matrix remodeling were initially included. These were screened for differential expression in GSE30784 and further narrowed in GSE41613 by combining older-age-related trends and survival information. Ultimately, 5 key genes were identified: *ANGPT2*, *HIF1A*, *VEGFC*, *TGFB1*, and *MMP9*. In addition, *IL1B* and *IL6* were retained as auxiliary genes for supplementary analysis.

Table 1. Basic information of the included GEO datasets

Dataset	Platform		Sample Type	Sample Size	Main Use
GSE30784	GPL570	OSCC, dysplasia, normal tissue	229	Candidate-gene differential expression screening; immune infiltration analysis	
GSE41613	GPL570	OSCC	97	Older-age-related trend analysis; prognostic analysis	
GSE25099	GPL5175	OSCC, normal tissue	79	External expression validation	
GSE37991	GPL6883	Paired tumor/non-tumor tissue	80	Paired validation	

GSE30784 was used for candidate-gene differential expression screening and immune infiltration analysis, GSE41613 for older-age-related trend and prognostic analysis, GSE25099 for external expression validation, and GSE37991 for paired validation between tumor tissues and adjacent non-tumor tissues. GEO: Gene Expression Omnibus; OSCC: oral squamous cell carcinoma.

Table 2. Repair-related candidate genes and the rationale for their inclusion

Gene	Main Biological Function	Repair-Related Category	Rationale for Inclusion
<i>HIF1A</i>	Regulation of hypoxic response	Hypoxic response	Related to ischemia-reperfusion injury and tissue hypoxia
<i>ANGPT2</i>	Angiogenesis and vascular remodeling	Angiogenesis	Involved in local vascular instability and remodeling
<i>VEGFC</i>	Angiogenesis/lymphangiogenesis	Angiogenesis	Related to reconstruction of local microcirculation
<i>TGFB1</i>	Repair regulation and immune modulation	Repair regulation	Participates in tissue repair and immune homeostasis
<i>MMP9</i>	Matrix degradation and remodeling	Matrix remodeling	Related to wound remodeling and healing environment
<i>IL1B</i>	Inflammatory mediator	Inflammatory response	Related to the local inflammatory microenvironment
<i>IL6</i>	Inflammatory mediator	Inflammatory response	Related to chronic inflammation and tumor immunity

Candidate genes were mainly derived from the literature on flap survival, ischemia-reperfusion injury, wound healing, angiogenesis, inflammatory response, and matrix remodeling. Based on their biological functions and repair-related mechanistic characteristics, the genes were categorized into hypoxic response, angiogenesis, inflammatory response, repair regulation, and matrix remodeling. ANGPT2: angiopoietin 2; HIF1A: hypoxia inducible factor 1 subunit alpha; IL1B: interleukin 1 beta; IL6: interleukin 6; MMP9: matrix metalloproteinase 9; TGFB1: transforming growth factor beta 1; VEGFC: vascular endothelial growth factor C.

Age-related Immune Infiltration and SIF Prognosis

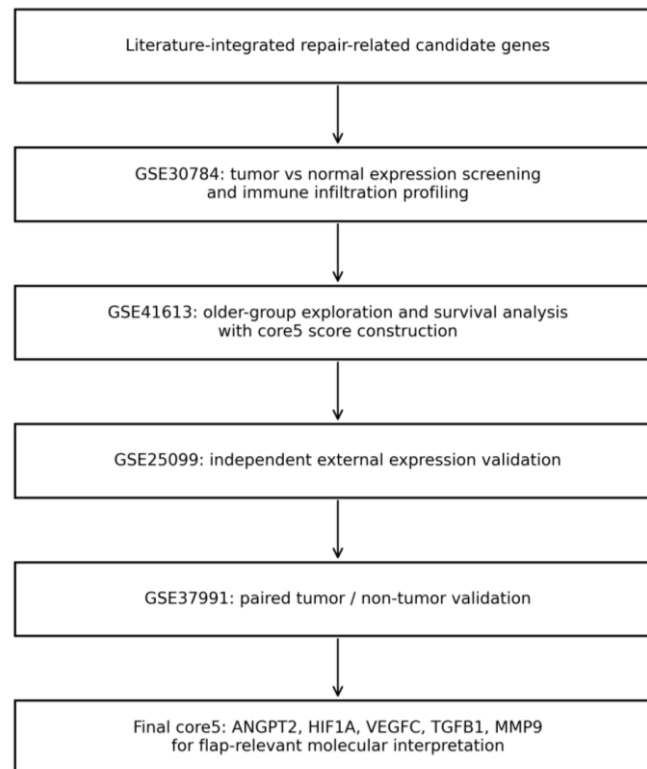


Figure 1. Study flowchart. Candidate genes related to flap repair were first screened through literature integration. Differential expression screening and immune infiltration profiling were performed using GSE30784. Older-age-related trend analysis and survival analysis were conducted using GSE41613. External validation and paired validation were performed using GSE25099 and GSE37991, respectively. Finally, *ANGPT2*, *HIF1A*, *VEGFC*, *TGFB1*, and *MMP9* were identified as key repair-related genes potentially associated with the local repair microenvironment relevant to submental island flap reconstruction.

Table 3. Screening results for the five key genes

Gene	GSE30784 Tumor/Normal Difference	GSE41613 Prognostic Relevance	GSE25099 External Validation	GSE37991 Paired Validation	Included in Final Analysis
<i>ANGPT2</i>	Yes	Yes	Yes	Yes	Yes
<i>HIF1A</i>	Yes	Yes	Yes	Yes	Yes
<i>VEGFC</i>	Yes	Yes	Yes	Yes	Yes
<i>TGFB1</i>	Yes	Yes	Yes	Yes	Yes
<i>MMP9</i>	Yes	Yes	Yes	Yes	Yes
<i>IL1B</i>	Yes	Weak/No	Yes	Yes	Auxiliary analysis
<i>IL6</i>	Yes	Weak/No	Yes	Yes	Auxiliary analysis
<i>CD163</i>	Yes	Weak/No	Yes	No	Not included

The screening criteria for key genes included significant differential expression between tumor and normal tissues in GSE30784, prognostic relevance in GSE41613, and consistent expression directions in both GSE25099 and GSE37991. *IL1B* and *IL6* were retained as auxiliary genes for supplementary analysis, whereas *CD163* was not included in the final analysis because of insufficient stability in external validation. CD163: cluster of differentiation 163.

Key Genes Are Aberrantly Overexpressed in Oral Cancer Tissues

In GSE30784, a total of 229 samples were included, comprising 167 tumor tissues, 45 normal oral tissues, and 17 dysplastic tissues. Expression analysis of the 5 key genes showed that *ANGPT2*, *HIF1A*, *VEGFC*, *TGFBI*, and *MMP9* were all expressed at significantly higher levels in tumor tissues than in normal tissues (Figure 2). These results suggest that the 5 key genes display stable abnormal expression patterns in oral cancer. Among them, *HIF1A* mainly represents abnormal hypoxic response, *ANGPT2* and *VEGFC* reflect alterations in angiogenesis and vascular remodeling, *TGFBI* is related to dysregulated repair control and immune homeostasis, and *MMP9* is closely associated with matrix degradation and tissue remodeling. Taken together, these age-related findings suggest that oral cancer may harbor a molecular microenvironment potentially unfavorable to local tissue repair, particularly in the context of older-age-related trends.

Combined Expression of Key Genes Is Associated with Poor Prognosis

In GSE41613, 97 OSCC samples were included. By integrating follow-up time, survival status, and the expression of the 5 key genes, a combined expression

score was constructed, and the cohort was divided into a high-score group and a low-score group using the median as the cut-off. Kaplan-Meier survival analysis showed that the probability of overall survival was lower in the high-score group than in the low-score group (Figure 3), suggesting that this molecular signature is associated with poorer prognosis. Further univariate Cox analysis indicated that *ANGPT2*, *HIF1A*, *VEGFC*, *TGFBI*, and *MMP9* all demonstrated a certain degree of prognostic relevance, with detailed results shown in Table 4. Because the age information in this cohort was presented as age ranges, the findings are more appropriately interpreted as reflecting an older-age-related trend rather than a strict age-stratified result. Overall, however, the results suggest that older-age-related trends in oral cancer may be associated with more unfavorable repair-related molecular characteristics.

External Validation of Key Genes in Independent Cohorts

The results showed that *ANGPT2*, *HIF1A*, *VEGFC*, *TGFBI*, and *MMP9* exhibited exactly the same direction of expression change as in GSE30784, all showing varying degrees of upregulation in tumor tissues (Figure 4). The corresponding validation results are summarized in Table 5.

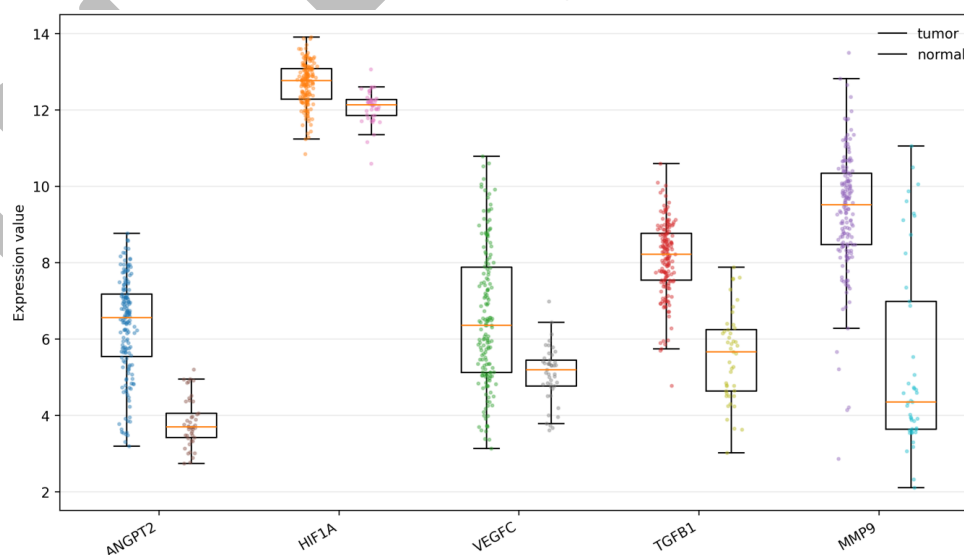


Figure 2. Differential expression of the five key genes in GSE30784. Expression differences of *ANGPT2*, *HIF1A*, *VEGFC*, *TGFBI*, and *MMP9* between oral cancer tissues and normal oral tissues in GSE30784 are shown. The results showed that these five key genes were overall upregulated in tumor tissues. Differences between groups were evaluated using the Wilcoxon rank-sum test, with multiple-testing correction applied where applicable.

Age-related Immune Infiltration and SIF Prognosis

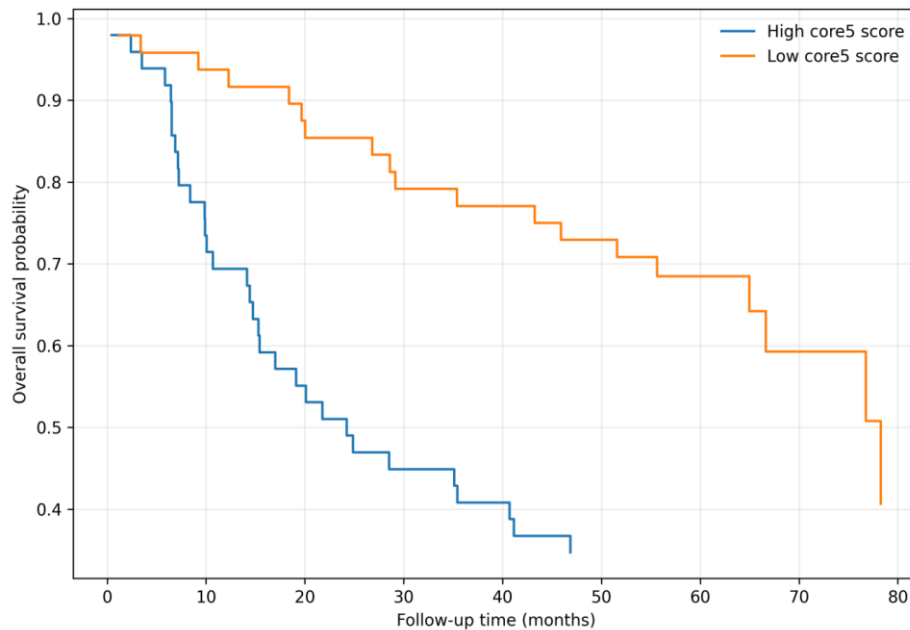


Figure 3. Survival analysis of the combined expression score of the five key genes in GSE41613. A score was constructed based on the combined expression of *ANGPT2*, *HIF1A*, *VEGFC*, *TGFB1*, and *MMP9* in GSE41613, and patients were divided into high-score and low-score groups according to the median. Kaplan-Meier curves showed that the high-score group had a lower overall survival probability, suggesting that the combined expression signature of these key genes is associated with poor prognosis. Because age information in this cohort was interval-based, the result should be interpreted as an older-age-related trend rather than a strict age-stratified conclusion.

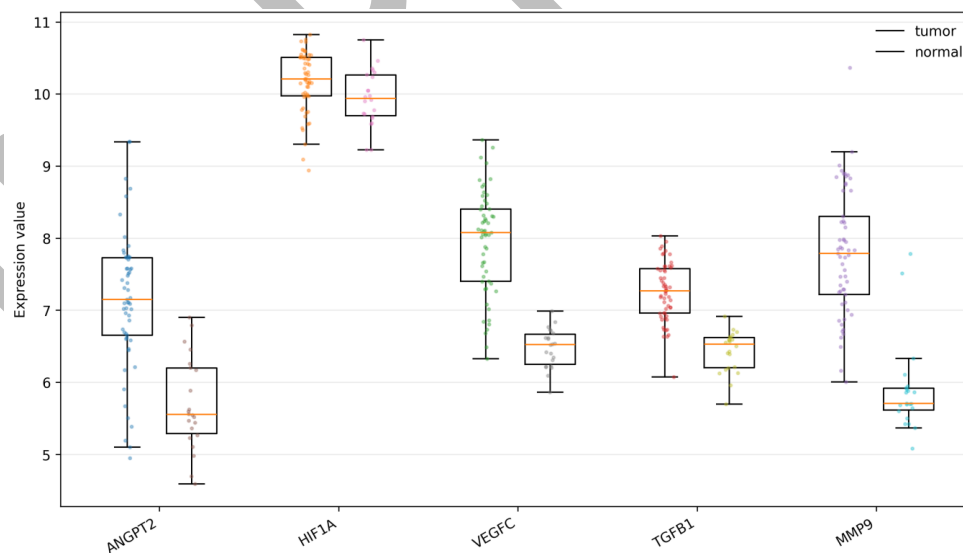


Figure 4. External validation of the five key genes in GSE25099. The expression of *ANGPT2*, *HIF1A*, *VEGFC*, *TGFB1*, and *MMP9* was validated in the independent cohort GSE25099. The results showed that the direction of expression of these five key genes in tumor tissues was consistent with that in the primary analysis cohort.

Table 4. Univariate Cox regression analysis of key genes in GSE41613

Gene	HR	95% CI	p
Combined expression score of the 5 key genes	1.801	1.172–2.767	0.00726
<i>ANGPT2</i>	2.065	1.402–3.042	0.00024
<i>HIF1A</i>	4.404	1.978–9.805	0.000283
<i>VEGFC</i>	1.466	1.182–1.818	0.000503
<i>TGFB1</i>	1.661	1.118–2.468	0.0119
<i>MMP9</i>	1.347	1.023–1.774	0.034
<i>TIMP2</i>	1.411	0.966–2.063	0.075
<i>IL6</i>	1.172	0.980–1.402	0.0817
<i>IL1B</i>	1.164	0.974–1.391	0.0947
<i>FGF2</i>	0.421	0.141–1.264	0.123
<i>NFE2L2</i>	0.691	0.430–1.109	0.126
<i>PDGFB</i>	1.417	0.824–2.436	0.207
<i>VEGFA</i>	1.141	0.879–1.482	0.32
<i>HGF</i>	0.821	0.498–1.353	0.438
<i>MRC1</i>	1.100	0.824–1.468	0.518
<i>HMOX1</i>	1.112	0.795–1.554	0.536
<i>IL10</i>	1.366	0.437–4.267	0.592
<i>CD163</i>	1.067	0.841–1.356	0.592
<i>CCL2</i>	1.052	0.807–1.370	0.709
<i>CASP3</i>	0.883	0.381–2.044	0.771
<i>TNF</i>	0.967	0.607–1.540	0.888

Univariate Cox regression analysis was used to evaluate the relationship between gene expression and overall survival. HR > 1 indicates that higher expression of the gene is associated with poorer prognosis. The combined expression score of the five key genes is listed at the top and highlighted because it represents the main prognostic signature of this study.

ANGPT2: angiopoietin 2; CASP3: caspase 3; CCL2: C-C motif chemokine ligand 2; CD163: cluster of differentiation 163; CI: confidence interval; FGF2: fibroblast growth factor 2; HGF: hepatocyte growth factor; HIF1A: hypoxia inducible factor 1 subunit alpha; HMOX1: heme oxygenase 1; HR: hazard ratio; IL10: interleukin 10; IL1B: interleukin 1 beta; IL6: interleukin 6; MMP9: matrix metalloproteinase 9; MRC1: mannose receptor C-type 1; NFE2L2: NFE2-like bZIP transcription factor 2; PDGFB: platelet derived growth factor subunit B; TGFB1: transforming growth factor beta 1; TIMP2: TIMP metalloproteinase inhibitor 2; TNF: tumor necrosis factor; VEGFA: vascular endothelial growth factor A; VEGFC: vascular endothelial growth factor C.

Table 5. Expression validation results of the five key genes in external validation cohorts

Gene	GSE25099 Direction	GSE25099 FDR	GSE37991 Direction	GSE37991 FDR	Consistency
<i>ANGPT2</i>	Upregulated	1.44×10^{-7}	Upregulated	7.28×10^{-12}	Consistent
<i>HIF1A</i>	Upregulated	1.37×10^{-2}	Upregulated	5.06×10^{-9}	Consistent
<i>MMP9</i>	Upregulated	1.02×10^{-9}	Upregulated	7.28×10^{-12}	Consistent
<i>TGFB1</i>	Upregulated	7.00×10^{-10}	Upregulated	2.33×10^{-9}	Consistent
<i>VEGFC</i>	Upregulated	7.00×10^{-10}	Upregulated	4.85×10^{-11}	Consistent

GSE25099 served as the independent external validation cohort, whereas GSE37991 served as the paired validation cohort of tumor tissues and adjacent non-tumor tissues. The consistency conclusion was based on whether the direction of gene expression was the same in the two cohorts. ANGPT2: angiopoietin 2; FDR: false discovery rate; HIF1A: hypoxia inducible factor 1 subunit alpha; MMP9: matrix metalloproteinase 9; TGFB1: transforming growth factor beta 1; VEGFC: vascular endothelial growth factor C.

Key Genes Are Closely Associated with Immune Infiltration Characteristics

To analyze the relationship between the key genes and the immune microenvironment, correlations between *ANGPT2*, *HIF1A*, *VEGFC*, *TGFB1*, and *MMP9* and multiple immune infiltration indices were further examined. The results showed that *ANGPT2*, *HIF1A*, *VEGFC*, and *TGFB1* were overall positively correlated with myeloid inflammatory features, whereas *MMP9* showed a more prominent correlation with M2 macrophages (Figure 6). Meanwhile, some key genes showed relatively weaker associations with CD8⁺ T-cell and NK-cell infiltration, suggesting that they may be associated with an immunosuppressive immune

infiltration pattern. These findings indicate that the 5 key genes are not merely related to tumor-associated expression differences but are also associated with remodeling of the local immune infiltration landscape in oral cancer. Considering the biological functions of these genes, these results suggest a possible association between older-age-related molecular features and a linked pattern characterized by enhanced hypoxic response, abnormal angiogenesis, activation of myeloid inflammation, and imbalanced matrix remodeling, which may be relevant to the local repair microenvironment and the potential prognosis of SIF reconstruction.

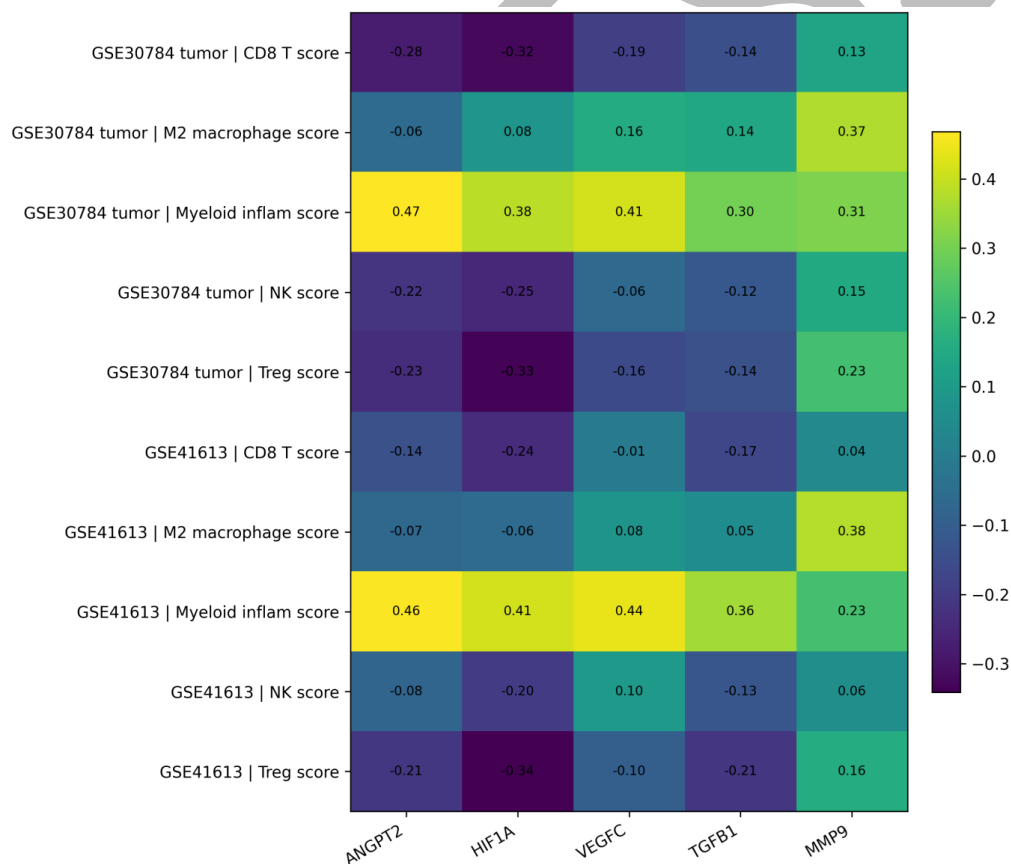


Figure 6. Correlation analysis between the five key genes and immune infiltration features. The heatmap shows Spearman correlation coefficients between *ANGPT2*, *HIF1A*, *VEGFC*, *TGFB1*, *MMP9* and immune infiltration indicators in GSE30784 and GSE41613. Rows represent immune infiltration features, including CD8⁺ T-cell score, M2 macrophage score, myeloid inflammatory score, NK-cell score, and Treg score. Columns represent the five key genes. Positive values indicate positive correlations, whereas negative values indicate negative correlations. *ANGPT2*, *HIF1A*, *VEGFC*, and *TGFB1* showed overall positive correlations with myeloid inflammatory features, whereas *MMP9* showed a more prominent correlation with M2 macrophage infiltration. These correlation results indicate associations rather than direct causal or regulatory relationships.

Summary of Results

By integrating literature review, screening in the primary cohort, prognostic analysis, external validation, and immune infiltration analysis, this study ultimately identified *ANGPT2*, *HIF1A*, *VEGFC*, *TGFB1*, and *MMP9* as 5 key repair-related genes in oral cancer with older-age-related trends. These molecules showed stable abnormal expression across multiple GEO cohorts and were associated with poorer prognosis and immune microenvironment imbalance, suggesting a molecular basis potentially unfavorable to wound healing and flap survival. These findings may provide a reference for patient selection and perioperative management in SIF reconstruction, particularly for oral cancer patients with older-age-related repair and immune microenvironment alterations.

DISCUSSION

Based on public databases, this study investigated age-related immune infiltration characteristics in oral cancer and, by integrating literature on flap repair, identified 5 key genes, namely *ANGPT2*, *HIF1A*, *VEGFC*, *TGFB1*, and *MMP9*. These genes exhibited stable abnormal expression across multiple independent cohorts and showed certain associations with poor prognosis and immune microenvironment remodeling. Overall, these age-related trends suggest that oral cancer patients of older age may harbor a molecular background potentially unfavorable to local tissue repair. This background appears not to be limited to tumor proliferation itself but may involve multiple processes such as enhanced hypoxic response, abnormal angiogenesis, activation of myeloid inflammation, and imbalanced matrix remodeling. Accordingly, the present study interprets these key genes as potential clues linking tumor biology with the prognosis of SIF reconstruction, rather than simply regarding them as general tumor markers.

First, from the perspective of the tumor immune microenvironment, the present findings are biologically plausible. OSCC is not merely an accumulation of cancer cells, but a complex ecosystem composed of tumor cells, immune cells, tumor-associated fibroblasts, endothelial cells, and extracellular matrix. Previous studies have shown that the TIME of OSCC broadly involves T cells, macrophages, dendritic cells, natural killer cells, and various inflammatory mediators, which

interact through cytokine and metabolic networks and ultimately affect tumor invasion, metastasis, and clinical outcomes.⁵ On this basis, regulatory T cells have been considered closely related to the immunosuppressive state of OSCC, and their abnormal enrichment may weaken antitumor immune responses and affect prognosis.¹⁶ Tumor-associated macrophages, especially those polarized toward the M2 phenotype, are even more closely related to angiogenesis, immune escape, and tumor progression.¹⁷ A systematic review and meta-analysis further showed that tissue-infiltrating immune cells such as CD163-positive M2 macrophages and CD57-positive NK cells carry relatively clear prognostic implications in oral cancer.¹⁸ In the present study, the 5 key genes were clearly related to myeloid inflammatory features, M2 macrophage infiltration, and certain changes in the immunosuppressive microenvironment, indicating that our findings do not represent isolated expression abnormalities but rather fit within the established theoretical framework of the TIME in OSCC.

Second, advanced age may be an important background factor driving the linkage among tumor progression, immune imbalance, and limited repair. Elderly patients are not characterized merely by chronological aging; they are often accompanied by systemic alterations such as immunosenescence and inflammaging, including decreased T-cell function, persistent low-grade chronic inflammation, disruption of the balance between innate and adaptive immunity, and reduced responsiveness to tissue-repair stimuli. Previous reviews have pointed out that immunosenescence and inflammaging jointly influence cancer development and treatment response, reflecting the integrated consequences of long-term remodeling of the host immune system.⁴ This background is particularly important in oral cancer. The oral region is chronically exposed to mechanical irritation, microbial burden, inflammatory reactions, and multiple carcinogenic factors. If patients of older age additionally experience local hypoxia, malnutrition, vascular aging, and reduced tissue regenerative capacity, they may be more likely to develop a local environment unfavorable to postoperative repair. In GSE41613, the present study observed that the older-age-related trend group displayed more pronounced unfavorable molecular features. Although the age information in that dataset was presented in intervals and therefore cannot fully

replace strict age stratification, the directional result still suggests that older-age-related prognostic risk may derive not only from tumor stage or general performance status but also from deeper immune and repair-related molecular imbalance.

Looking at the composition of the key genes, the 5 genes identified in this study primarily highlight the hypoxia-angiogenesis axis. Tumor hypoxia is 1 of the typical features of the OSCC microenvironment. As the core transcription factor of the hypoxic response, HIF-1 α (encoded by *HIF1A*) participates not only in glycolysis, cell survival, and invasive behavior but is also closely associated with poor prognosis in patients with OSCC.¹¹ In the present study, *HIF1A* remained upregulated in tumor tissues and was consistent with a poorer survival trend, suggesting that hypoxic-response-related features in oral cancer with older-age-related trends may be associated not only with malignant tumor progression but also with a microenvironment unfavorable to repair. Closely associated with this is abnormal angiogenesis and vascular remodeling. Angiopoietin-2 (encoded by *ANGPT2*) can destabilize blood vessels and promote neovascular remodeling, and has been demonstrated in OSCC to be associated with enhanced angiogenesis and reduced overall survival.¹² High expression of VEGF family members, especially VEGF-C, has been linked in OSCC to lymphatic metastasis, tumor progression, and poor prognosis.¹³ Both *ANGPT2* and *VEGFC* entered the final set of key genes in this study, indicating that oral cancer with older-age-related molecular features may not simply exhibit insufficient blood supply but rather a pattern of hypoxia-related abnormal and inefficient vascular reconstruction. For the tumor itself, such a state implies advantages in invasion and metastasis; for postoperative wounds and flaps, it may mean unstable perfusion quality, lower efficiency of microcirculatory reconstruction, and an unfavorable healing environment. In other words, the microenvironment formed by tumor hypoxia and abnormal angiogenesis may constitute an important molecular basis affecting the prognosis of SIF reconstruction.

Another major line running parallel to hypoxia and angiogenesis is abnormal repair regulation and matrix remodeling. *TGFBI* and *MMP9*, identified in this study, are particularly informative in this respect. TGF- β 1 is a key molecule involved in wound healing, inflammatory regulation, fibrosis, and immune balance; yet in the tumor environment it often exerts dual roles in

promoting both invasion and immune escape. MMP-9 mainly participates in basement-membrane destruction, extracellular matrix degradation, cell migration, and local tissue reconstruction. Previous studies have shown that, in OSCC, MMP-9 is closely associated with invasion pattern and metastasis, whereas TGF- β 1 is also related to tumor biological behavior.¹⁴ This means that *TGFBI* and *MMP9* do not simply represent either bad tumor biology or good repair but rather a complex dynamic balance: moderate repair-related signaling may favor healing, but when such signaling is chronically and abnormally activated in the tumor background, it often turns into a driver of invasion, immune suppression, and aberrant remodeling. In the present study, *TGFBI* and *MMP9* were stably upregulated across multiple cohorts and showed importance at both the prognostic and immune-infiltration levels, suggesting that the local tissues of elderly patients with oral cancer may be in a state of persistent remodeling with suboptimal quality. For postoperative wounds, this means not the absence of repair responses but rather repair responses that may be imbalanced, excessively consuming, or misdirected, thereby adversely affecting flap attachment, re-establishment of blood supply, and long-term stable healing.

Although *IL1B* and *IL6* were not included in the final set of 5 key genes, they still deserve discussion as auxiliary genes. IL-1 β is a typical pro-inflammatory factor that can participate in tumor-associated chronic inflammation and also promote malignant transformation and increased invasiveness of cells. Previous studies in oral cancer models have confirmed that elevated IL-1 β is associated with a greater degree of malignant transformation and enhanced tumor aggressiveness.¹⁹ IL-6 is 1 of the most representative cytokines in inflammaging and tumor-associated chronic inflammation. Earlier studies had already observed elevated levels of IL-1 β , IL-6, and TNF- α in the peripheral blood of patients with oral cancer, suggesting systemic significance of the inflammatory network in the development of oral cancer.²⁰ In the present study, *IL1B* and *IL6* still showed a certain degree of consistency at the expression and external-validation levels, but their prognostic stability was weaker than that of *ANGPT2*, *HIF1A*, *VEGFC*, *TGFBI*, and *MMP9*. This result itself is informative: in elderly patients with oral cancer, although elevated inflammatory activity is important, what truly determines the tendency toward unfavorable repair may not be a single isolated

inflammatory factor but rather a composite microenvironment formed by the interaction of inflammation, hypoxia, angiogenesis, and matrix remodeling. Therefore, retaining *IL1B* and *IL6* as auxiliary molecules rather than directly including them among the final key genes is more consistent with the data characteristics and with the actual state of research on the oral-cancer microenvironment.

From the perspective of repair biology, the key genes identified in this study are highly consistent with the known mechanisms of flap survival. A recent systematic review on flap ischemia/reperfusion injury showed that, in models related to microsurgical reconstruction, the most common molecular alterations were concentrated in *TNF- α* , *IL-6*, *IL-1 β* , *caspase-3*, *VEGF*, and *HIF-1 α* , corresponding to inflammatory cascades, apoptosis, hypoxic adaptation, and angiogenesis.⁹ Another systematic review and meta-analysis on gene therapy for improving flap survival pointed out that evidence is most concentrated on VEGF-related interventions, whereas FGF, PDGF, HGF, and other molecules may also improve flap survival by enhancing blood supply and promoting neovascularization.¹⁰ Although this study did not directly measure these molecules in tissues from patients undergoing SIF reconstruction, from a mechanistic point of view *ANGPT2*, *HIF1A*, *VEGFC*, *TGFB1*, and *MMP9* cover precisely several of the most important aspects of flap repair: hypoxia sensing, vascular reconstruction, inflammatory regulation, and matrix remodeling. Therefore, interpreting them as potential clues associated with the prognosis of SIF reconstruction is justified. Particularly in elderly patients, abnormalities in these molecules may imply that, after surgical injury, local tissues are more likely to enter a vicious cycle characterized by insufficient perfusion, prolonged inflammation, and imbalanced repair, thereby increasing the risk of marginal flap necrosis, delayed healing, or limited functional recovery.

When considered together with the clinical characteristics of the SIF itself, the conclusions of this study also carry relatively clear translational value. Previous studies have shown that the SIF has advantages in oral soft-tissue reconstruction, including relative ease of operation, a concealed donor site, shorter operative time, and lower cost, and that in some cases it can achieve satisfactory functional and oncologic outcomes.⁶ Studies in young, middle-aged, and elderly patients with oral tongue squamous cell carcinoma have

further shown that the facial-submental island flap has a high success rate across different age groups and also performs well in elderly patients.⁷ This suggests that the SIF is not a technical choice whose outcome is determined solely by the surgical method, but rather a reconstructive strategy that is highly dependent on the patient's baseline condition and the local microenvironment. If, in elderly patients, molecular features such as abnormal hypoxic response, enhanced aberrant angiogenesis, elevated myeloid inflammation, and imbalanced matrix remodeling can be identified preoperatively, clinicians may be able to make more targeted decisions regarding case selection, perioperative infection and inflammation control, nutritional support, monitoring of operative-site perfusion, and postoperative follow-up. Therefore, although this study did not directly observe actual flap failure events, its significance is not limited to theory but may also provide a basis for identifying which elderly patients with oral cancer require more cautious assessment of SIF reconstructive risk.

This study also has several limitations that should be clearly acknowledged. First, it is based on public databases, and the predominant pathological type among the included samples was in fact OSCC. Therefore, although the manuscript uses the broader term oral cancer, interpretation of the results should mainly be grounded in oral squamous cell carcinoma. Second, public databases lack actual SIF-related surgical variables, such as partial flap necrosis, venous crisis, wound dehiscence, swallowing recovery, donor-site complications, and functional scores. Consequently, what this study refers to as an impact on the prognosis of SIF reconstruction should be more accurately understood as a potential influence through alteration of the local repair microenvironment, rather than as direct causal proof. Third, age information was not presented as exact age in all datasets, and analysis of older-age-related trends is therefore more suitable as directional evidence than as a substitute for strict age-stratified research. Fourth, due to the retrospective nature of public GEO datasets, detailed and harmonized clinicopathological information, including exact age, comorbidities, treatment history, and reconstructive outcomes, was not fully available across all cohorts. Fifth, because this study was based on transcriptomic correlation analyses, the observed associations should not be interpreted as direct causal or regulatory relationships without further experimental validation.

Finally, the study still lacks further validation using real-world cases and tissue samples, especially evidence directly linking the expression of key genes to postoperative outcomes of the SIF on a case-by-case basis. Future studies using real-world oral cancer tissues and clinical cohorts with documented SIF reconstructive outcomes are needed to validate the biological and clinical relevance of these genes.

Despite these limitations, the present study still has a certain degree of innovation and practical significance. First, it did not screen molecules solely from the perspective of tumor prognosis but rather incorporated elderly status, immune infiltration, repair-related genes, and SIF prognosis into a unified analytical framework, emphasizing the continuity between tumor biology and reconstructive repair. Second, the identified key genes were not limited to general differential expression findings, but were supported across the primary cohort, external cohort, and paired cohort, making them relatively stable. Third, the most direct clinical implication of the findings is that future risk assessment for reconstruction in elderly oral cancer may need to consider not only defect size, vascular pedicle length, surgical method, and general comorbidity status but also differences in the local molecular repair microenvironment. If future real-world studies confirm direct associations between these key genes and SIF survival, healing quality, and long-term functional recovery, this molecular set may further develop into an auxiliary assessment index for reconstructive decision-making in elderly patients with oral cancer.

In summary, the present study suggests that older-age-related trends in oral cancer may be associated with an unfavorable repair microenvironment jointly shaped by enhanced hypoxic response, abnormal angiogenesis, persistent inflammatory activation, and imbalanced matrix remodeling. *ANGPT2*, *HIF1A*, *VEGFC*, *TGFB1*, and *MMP9*, as the most representative key genes within this pattern, can help explain both tumor biological progression and potential differences in the prognosis of SIF reconstruction from a molecular perspective. Future validation and functional studies that integrate these key molecules with real-world cases will help promote a transition from experience-based reconstruction choices to biologically guided individualized reconstructive strategies for oral cancer patients with older-age-related repair and immune microenvironment alterations.

STATEMENT OF ETHICS

Not applicable. This study was based on public datasets and did not involve direct recruitment of human participants or animal models.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

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