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Effect of Indirect Co-Culture With Gingival Mesenchymal Stem Cells on Cytokine Secretion in Primary Oral Squamous Cell Carcinoma Cells

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Conflict of interest: None declared

Background: This study evaluated whether indirect paracrine exposure to gingival mesenchymal stem cells (GMSCs) alters inflammatory cytokine signaling in low-passage primary epithelial-enriched cells derived from histopathologically confirmed oral squamous cell carcinoma (OSCC) tissue.

Material/Methods: Two experimental models were used: (1) treatment with 50% and 100% GMSC-conditioned medium (GMSC-CM), and (2) indirect Transwell co-culture with live GMSCs (0.4-μm pore size). Primary OSCC cultures were established from 3 independent histopathologically confirmed OSCC donor specimens and used at passage 1. GMSCs were isolated from healthy gingival tissue from 3 donors, expanded in culture, immunophenotypically verified, and used at passage 4. Each biological experiment was performed in triplicate. Outcomes assessed were cytokine secretion determined by cytokine bead array (CBA), and inflammatory and gene expression assessed by RT-qPCR, and we performed phase-contrast morphology computational/statistical analysis.

Results: GMSCs demonstrated a fibroblast-like spindle morphology with positive CD73/CD90 and negative CD34/CD45 expression, consistent with MSC identity. OSCC Control released high levels of pro-inflammatory cytokines, including IL-6, IL-8, MCP-1/CCL2, and TNF-α. RT-qPCR showed increased IL10 and CXCL10 and decreased TNF, CCL2, and IL6 following indirect GMSC exposure, consistent with altered inflammatory cytokine signaling in vitro. Spearman analyses showed strong associations between transcript levels and corresponding secreted cytokines.

Conclusions: Indirect GMSC signaling (Transwell co-culture or conditioned medium) attenuated pro-inflammatory cytokine secretion (IL-6, TNF-α, MCP-1/CCL2) and increased IL-10 and CXCL10/IP-10 expression in primary OSCC cells, indicating reshaping of inflammatory cytokine signaling in vitro. Functional tumor assays and immune-cell–inclusive models are needed to determine downstream effects on OSCC behavior and immune interactions.

Keywords: Carcinoma, Squamous Cell • Co-Culture Techniques • Cytokines • Gingiva • Mesenchymal Stem Cells • Oncology

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Introduction

Oral cancer, a leading cause of death, is more common in poorer nations. Because oral cancer is difficult to diagnose and treat quickly, its mortality and morbidity rates are high [1]. In the oral cavity, one of the most prevalent cancers is OSCC. Its high morbidity and mortality rates can be significantly decreased by prompt diagnosis and treatment. Early lesion diagnosis is not facilitated by traditional diagnostic techniques such as biopsy. At this stage, the use of tumor markers becomes crucial. In addition to providing information on the lesion's present state, tumor markers can also reveal whether the malignancy is still spreading [2].

The mesoderm is the source of mesenchymal stem cells, which are found in many human tissues, including bone marrow, adipose tissue, skin, peripheral blood, thymus, placenta, umbilical cord blood, tendons, and the heart [3]. Current methods make it easier to isolate, cultivate, and expand MSCs. These cells can differentiate into other mesenchymal cell types, including adipocytes, liver cells, tenocytes, chondrocytes, osteoblasts, and nerve cells, using a variety of in vitro induction approaches. Strong immunomodulatory effects and low immunogenicity both occur in vivo.

GMSCs are more readily available than other types of MSCs, can be acquired with comparatively minimally invasive techniques, and have sufficient genetic stability. Additionally, they can be used in autologous transplants, which do not require a matching donor search. Since 2009, when Zhang et al initially identified and thoroughly documented the isolation, characterization, and immunomodulatory capabilities of GMSCs, these cells have interested researchers worldwide due to their unparalleled benefits [4,5].

Biomarkers are quantifiable biological indicators that hold promise for monitoring and early detection. In OSCC, oral fluids such as saliva provide a non-invasive window into disease-associated biology. Multiple studies and systematic reviews have evaluated salivary cytokines, including IL-6, IL-8, TNF-α, IL-10, and related chemokines as candidate biomarkers for OSCC detection, risk stratification, and monitoring [6-10].

IL-10 is an anti-inflammatory cytokine produced by multiple cell types (including macrophages, lymphocytes, and epithelial cells) and has context-dependent roles in cancer. In many settings, IL-10 can contribute to immune suppression and tumor-immune evasion, although its effects are complex and vary with microenvironmental context [11].

Prior research has shown that gingival fibroblasts can differentiate into CAFs when exposed to OSCC-conditioned media (OSCC CM). Notably, cytokines like CXCL1 are essential for mediating this shift and establishing a positive feedback loop that improves interactions between tumors and the stroma [12].

Oral inflammatory fluids other than saliva, such as gingival crevicular fluid (GCF), contain a mix of substances from both the body and oral bacteria, including proteins, cytokines, antibodies, bacterial components, electrolytes, and enzymes [13-15]. These biofluids reflect local tissue inflammation and can complement the use of saliva-based biomarkers. The present study focused specifically on paracrine GMSC-OSCC interactions that can shape cytokine signaling within OSCC cells.

The pleiotropic cytokine IL-6 promotes tumor cell migration and invasion by activating the JAK/STAT3 signaling pathway, which in turn suppresses epithelial characteristics and induces the expression of mesenchymal markers [17,18]. Apart from its role in epithelial-mesenchymal transition (EMT), IL-6 also plays a role in immunological regulation, stromal remodeling, and angiogenesis, all of which promote tumor growth [19,20]. However, little is known about the exact cellular origins of IL-6 inside the TME, the processes controlling its release, and how it specifically affects the course of OSCC. A tumor marker is characterized as one that transmits data regarding the possible metastasis of an existing malignancy; this has a significant impact on tumor diagnosis [21]. An ideal tumor marker should accurately detect tumors without missing cases (false negatives), specifically identify the type of tumor, and help in early tumor detection [22].

There is little information in the literature about how indirect co-cultivation with GMSCs affects the release of cytokines in primary OSCC cells. To explore the wider therapeutic possibilities of GMSCs in tissue engineering, regenerative medicine, and treatment of inflammatory or immune-related disorders, we sought to physiologically characterize GMSCs and to assess their therapeutic use. Our objective was to determine the effects of indirect co-cultivation with GMSCs and GMSC-conditioned medium on the production of cytokines in primary OSCC cells.

Material and Methods

Study Design and Overview

Two models were used to assess how exposure to GMSCs affected the inflammatory and cytokine responses of primary OSCC cultures: (1) treatment with 50% and 100% GMSC-conditioned medium (GMSC-CM), and (2) indirect Transwell co-culture with live GMSCs using 0.4-μm pore inserts. OSCC cells cultured without GMSC exposure served as the control condition. The OSCC cultures used in this study were low-passage primary epithelial-enriched cultures derived from histopathologically confirmed malignant OSCC tissue. Three independent OSCC donor-derived primary cultures and 3 independent GMSC donor

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samples were included. Each experiment was performed in triplicate. Outcome measures included cytokine secretion by CBA, inflammatory gene expression by reverse transcription quantitative polymerase chain reaction (RT-qPCR), and computational/statistical analysis.

Ethics Approval and Consent

Use of human OSCC specimens and healthy gingival tissues was approved by the Research Ethics Committee at King Khalid University (HAPO-06-B-001) under Approval No. KKU-72-2025-21. All procedures were performed in accordance with institutional guidelines and the principles of the Declaration of Helsinki. Written informed consent was obtained from all tissue donors prior to sample collection. All specimens were de-identified before laboratory processing and analysis.

Human Tissue Sources and Replicates

Primary OSCC cultures were established from 3 independent surgically resected tumor specimens that were subsequently confirmed as malignant oral squamous cell carcinoma by histopathology. The clinicopathologic details corresponded to the same specimens used for primary culture establishment. Gingival tissues used for GMSC isolation were obtained from 3 healthy donors undergoing routine periodontal soft tissue procedures under aseptic conditions. OSCC primary cultures were used at passage 1, and GMSCs were used at passage 4. Biological replicates refer to independent donor-derived OSCC cultures, whereas technical replicates refer to replicate wells within the same experiment. Seeding density, timing, and medium volumes were kept constant across conditions.

Isolation and Culture of Primary OSCC Cells

Fresh tumor tissue was obtained from the same surgical specimens that were subsequently confirmed as malignant OSCC by histopathology. Immediately after excision, the tissue was placed in sterile phosphate-buffered saline (PBS) and transported directly to the laboratory for processing. Tumor specimens were washed with PBS containing antibiotic-antimycotic solution, minced aseptically with sterile surgical scissors, and subjected to enzymatic digestion using 0.4% collagenase I and 0.2% dispase II at 37°C for 30 minutes. The enzyme reaction was neutralized with fetal bovine serum (FBS), and the resulting cell suspension was passed through a sterile 70 µm cell strainer and centrifuged at 1800 rpm for 5 minutes.

The collected cell pellet was initially plated in DMEM supplemented with 20% FBS and antibiotic-antimycotic solution to facilitate primary attachment and early outgrowth. After initial establishment, the cultures were transferred to the Gibco™ Keratinocyte SFM (1X) Kit to support epithelial maintenance and minimize stromal overgrowth. Cell growth and morphology were monitored by inverted phase-contrast microscopy, and adherent cells were passaged using 0.25% trypsin-EDTA. Because these primary cultures had limited expansion capacity and could not be propagated as stable long-term cell lines, passage 1 cells were used for all experiments. Accordingly, the OSCC cultures are described here as low-passage primary epithelial-enriched cells derived from histopathologically confirmed malignant OSCC tissue.

The distinction between normal oral epithelial cells and OSCC-derived cells in this study was made primarily at the source-specimen level and by the culture strategy used. Cells derived directly from tumor specimens that were later confirmed as malignant OSCC by histopathology were used only at passage 1, and were maintained under epithelial-supportive keratinocyte serum-free culture conditions after initial attachment. This approach was intended to favor epithelial outgrowth and reduce stromal/fibroblastic overgrowth. Because no additional post-isolation molecular or immunophenotypic tumor-authentication assay was performed, the model is regarded as an epithelial-enriched low-passage primary culture derived from malignant OSCC tissue, rather than as a fully authenticated long-term OSCC cell line.

Isolation, Culture, and Validation of GMSCs

Healthy gingival tissues were obtained from 3 donors undergoing routine periodontal soft tissue procedures under aseptic conditions. The tissues were washed thoroughly with sterile PBS containing antibiotic-antimycotic solution, minced and macerated with a sterile blade, and subjected to brief enzymatic digestion using 0.25% trypsin-EDTA and 0.2% dispase II for 5 minutes to facilitate separation of the epithelial component. The epithelial fraction was discarded, and the remaining connective tissue component was further minced and incubated for 20 minutes at 37°C in the same enzyme mixture. Enzymatic activity was neutralized with FBS, and the digested tissue was passed through a sterile 70-µm cell strainer and centrifuged at 1800 rpm for 5 minutes.

The isolated cells were expanded in DMEM supplemented with 10% FBS and antibiotic-antimycotic solution at 37°C in 5% CO_2. The medium was changed twice weekly, and cell growth was monitored by phase-contrast microscopy. Adherent fibroblast-like cells were expanded by routine trypsinization using 0.25% trypsin-EDTA, and passage 4 GMSCs were used in all experiments.

GMSC identity was verified morphologically and immunophenotypically. The cells displayed typical spindle-shaped fibroblast-like morphology. For flow-cytometric verification, confluent GMSCs were dissociated, washed twice in PBS, and incubated for 30 minutes at 4°C with antibodies against CD73 and CD90 as mesenchymal stem cell markers and CD34 and CD45 as negative hematopoietic/non-MSC markers. Antibody-stained cells

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Table 1. RT-qPCR primer sequences.

Gene	Forward primer	Reverse primer
IL10	5'-TCT CCG AGA TGC CTT CAG CAG A-3'	5'-TCA GAC AAG GCT TGG CAA CCC A-3'
CXCL10	5'-GGT GAG AAG AGA TGT CTG AAT CC-3'	5'-GTC CAT CCT TGG AAG CAC TGC A-3'
IL1β	5'-CCA CAG ACC TTC CAG GAG AAT G-3'	5'-GTG CAG TTC AGT GAT CGT ACA GG-3'
IL4	5'-CCG TAA CAG ACA TCT TTG CTG CC-3'	5'-GAG TGT CCT TCT CAT GGT GGC T-3'
CCL2	5'-AGA ATC ACC AGC AGC AAG TGT CC-3'	5'-TCC TGA ACC CAC TTC TGC TTG G-3'
TNF	5'-CTC TTC TGC CTG CTG CAC TTT G-3'	5'-ATG GGC TAC AGG CTT GTC ACT C-3'
IL6	5'-AGA CAG CCA CTC ACC TCT TCA G-3'	5'-TTC TGC CAG TGC CTC TTT GCT G-3'
CXCL8	5'-GAG AGT GAT TGA GAG TGG ACC AC-3'	5'-CAC AAC CCT CTG CAC CCA GTT T-3'
IL2	5'-AGA ACT CAA ACC TCT GGA GGA AG-3'	5'-GCT GTC TCA TCA GCA TAT TCA CAC-3'
GAPDH	5'-GTC TCC TCT GAC TTC AAC AGC G-3'	5'-ACC ACC CTG TTG CTG TAG CCA A-3'

were washed twice with PBS, and 10 000 events per sample were acquired on an Attune NxT Flow Cytometer using isotype controls to discriminate positive and negative populations. The observed immunophenotype supported mesenchymal stromal/stem cell identity.

Preparation of GMSC-Conditioned Medium (GMSC-CM)

GMSC-conditioned medium was prepared from passage 4 GMSCs derived from 3 healthy donors. After plating, the cells were grown to approximately 70% confluence, washed twice with PBS, and incubated for 24 hours in serum-reduced DMEM containing 1% FBS. The conditioned medium was then collected, centrifuged at 1600 rpm for 5 minutes to remove cellular debris, filtered through a 0.22 µm membrane, aliquoted, and stored at -80°C until use. Fresh or singly thawed aliquots were mixed with OSCC culture medium to generate the 50% and 100% GMSC-CM treatment conditions.

Indirect Co-Culture

For indirect paracrine co-culture experiments, OSCC cells were seeded in the lower wells, and GMSCs were seeded in 0.4-µm pore size Transwell inserts positioned above the OSCC cultures. This configuration permitted the exchange of soluble factors while preventing direct cell-cell contact. Co-culture was maintained for 24 hours under standard incubator conditions. All donor-derived cultures were processed under standardized seeding density, timing, and medium-volume conditions, and each experimental condition was assessed in triplicate.

Morphological Observation

Using phase-contrast microscopy (10x magnification), cell morphology was investigated. Cell density, clumping, and shape changes were qualitatively recorded.

Cytokine Quantification

Culture supernatants were collected at the designated experimental endpoint and clarified by brief centrifugation. Concentrations of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, MCP-1/CCL2, and IP-10/CXCL10 were quantified using a CBA on the Attune NxT Flow Cytometer according to the manufacturer's instructions. Cytokine values were expressed as pg/mL for each condition. For each biological replicate, measurements from 3 technical replicate wells per condition were averaged before statistical analysis. Data are presented as mean±SD.

RNA Isolation and Reverse Transcription Quantitative PCR (RT-qPCR)

Total RNA was extracted from OSCC cells using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. RNA concentration and purity were measured using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). For cDNA synthesis, 1 µg of total RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. Quantitative PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Austin, TX, USA) on the QuantStudio 5 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

Primers for TNF, CXCL10, CCL2, IL1B, IL2, IL4, IL6, IL8, IL10, and GAPDH were obtained from published sequences or designed using NCBI Primer-BLAST. Primer sequences are presented in **Table 1**. Amplification efficiency was confirmed to be within the acceptable range of 90% to 110% by standard-curve analysis, and specificity was assessed by melt-curve analysis. No-template controls and no-reverse-transcriptase controls were included in each run. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method

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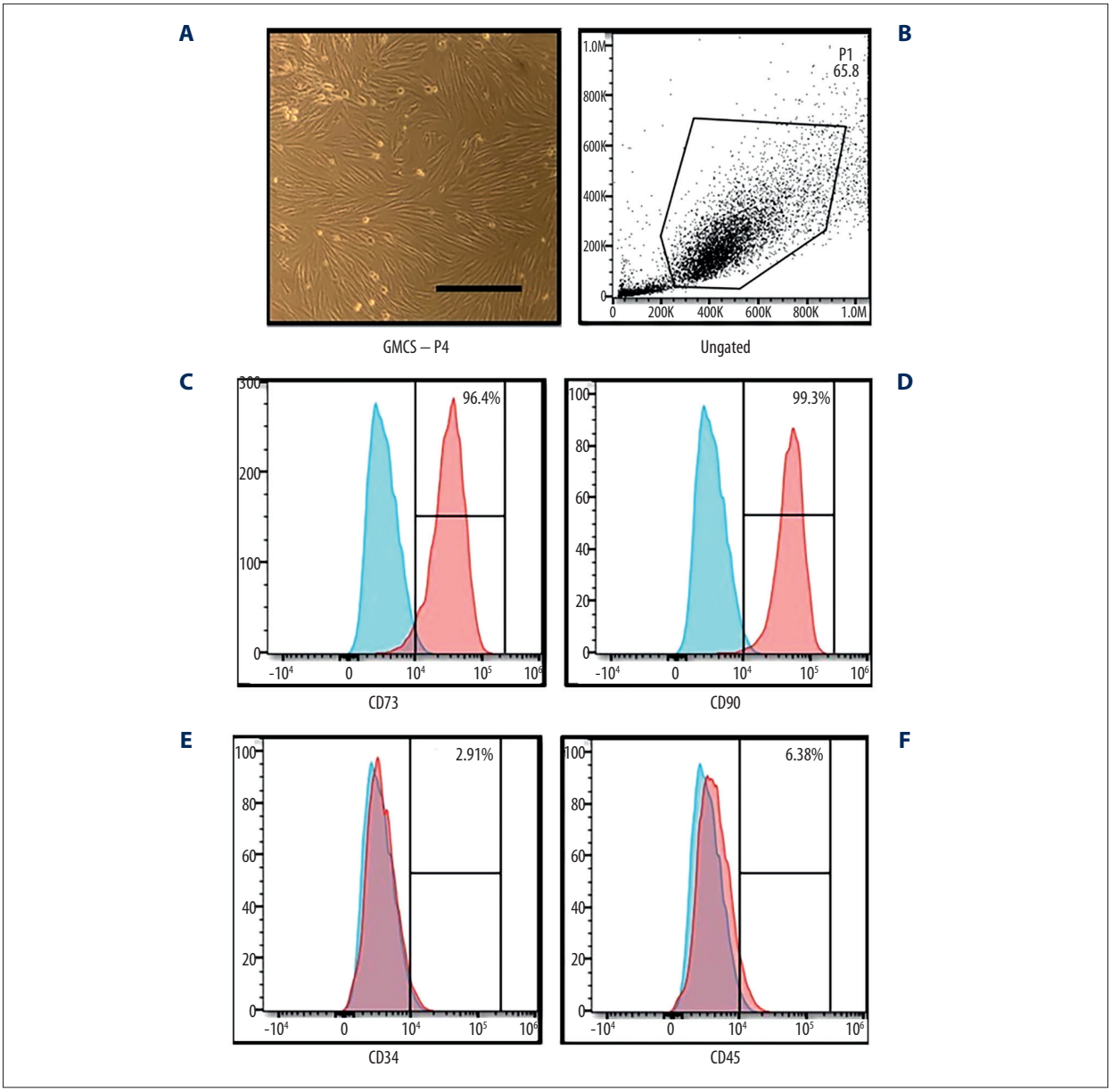

Figure 1. Morphological and immunophenotypic characterization of GMSCs. (**A**) Representative phase-contrast image of passage 4 GMSCs showing spindle-shaped fibroblast-like morphology. (**B**) Representative ungated flow-cytometry acquisition/scatter plot of the raw cell population, with the analysis gate indicated. (**C-F**) Representative histograms for CD73, CD90, CD34, and CD45.

after normalization to GAPDH, with the OSCC Control group used as the calibrator.

Computational and Statistical Analysis

All analyses were performed in R (v4.4.0) under WSL Ubuntu 22.04. Data were imported and cleaned using tidyverse (readr/dplyr/tidyr). Prior to parametric testing, distributional assumptions were assessed using Shapiro-Wilk tests and Q-Q plots; variance homogeneity was evaluated using Levene's (or Brown-Forsythe) tests. When assumptions were met, comparisons of control vs each treatment used Welch's *t* test (or paired *t* test when measurements were paired within the same donor, as specified in the replicate subsection). When assumptions were not met or the sample size was small, the Mann-Whitney U test (or Wilcoxon signed-rank test for paired data) was used. *P* values were adjusted for multiple comparisons using the Benjamini-Hochberg FDR procedure ($q<0.05$). Effect sizes (mean difference or log2 fold-change) are reported alongside adjusted *P* values. For correlation analysis, Spearman

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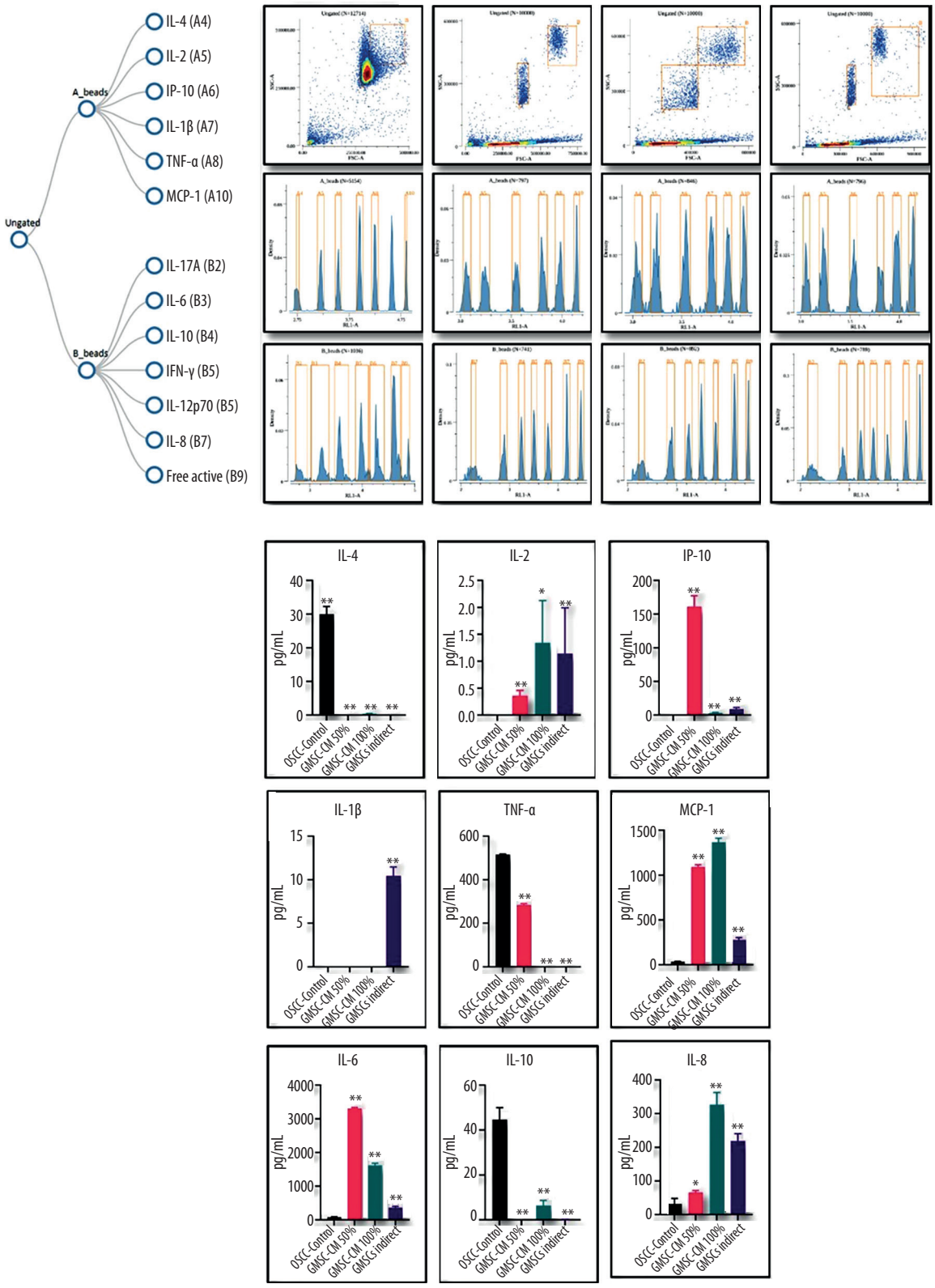

Figure 2. Cytokine profile modulation.

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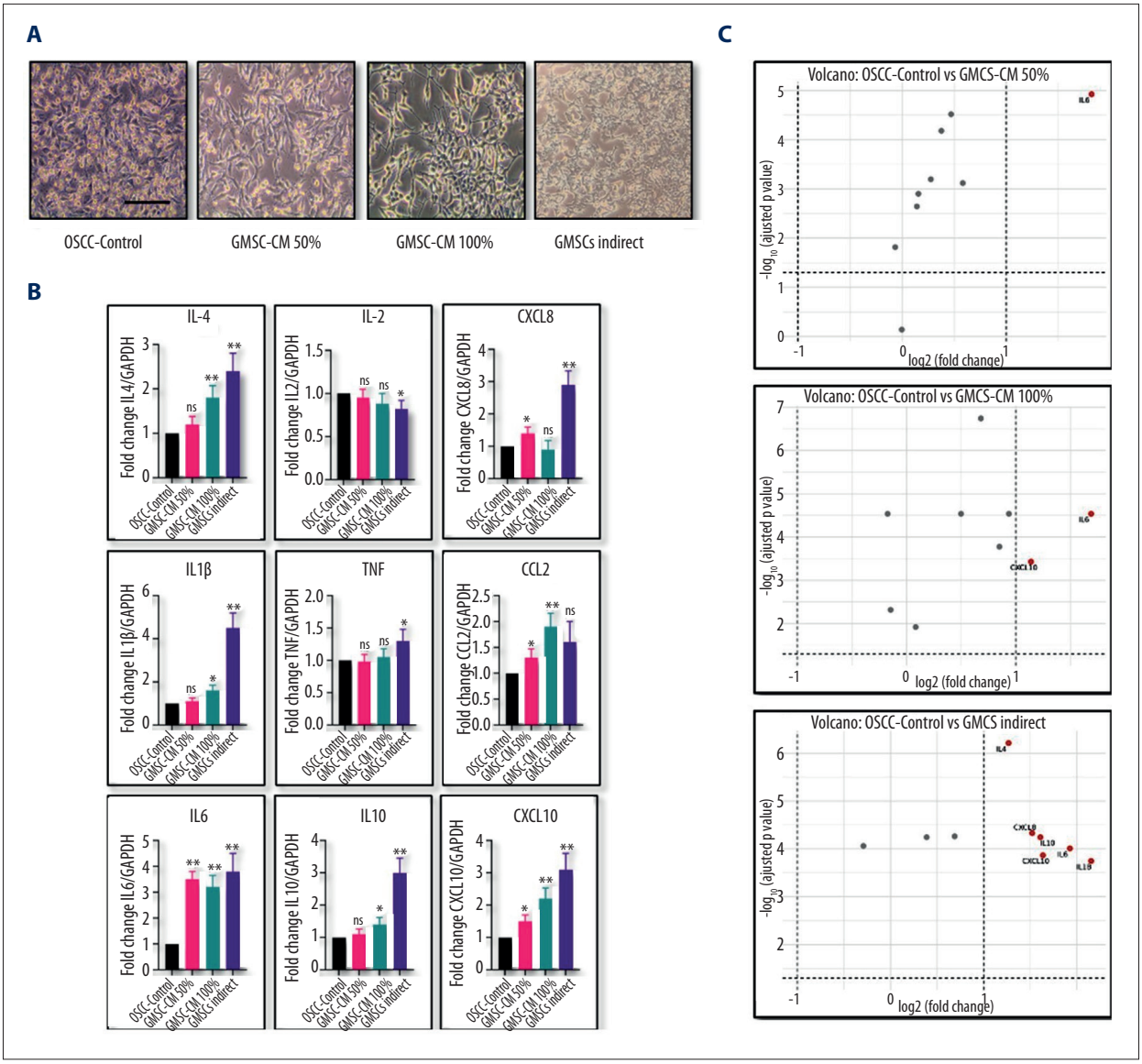

Figure 3. Gene expression pattern.

correlation coefficients (ρ) were calculated between matched cytokine protein and transcript-level measurements across conditions. For principal component analysis (PCA), cytokine and gene expression features were centered and scaled prior to dimensionality reduction. Graphical outputs were generated using ggplot2, ggpubr, pheatmap, and base R.

Results

GMSC Phenotype Confirmation

Primary GMSCs displayed the expected spindle-shaped fibroblast-like morphology in culture. Flow-cytometric immunophenotyping demonstrated positive expression of CD73 and CD90, together with negative expression of CD34 and CD45, supporting mesenchymal stromal/stem cell identity (**Figure 1**).

Cytokine Profile Modulation

High amounts of pro-inflammatory cytokines (IL-6, IL-8, MCP-1/CCL2, and TNF-α) were secreted by OSCC Control. After normalization to [viable cell count/total protein], these mediators were significantly reduced following treatment with GMSC-CM and indirect Transwell co-culture (adjusted $P<0.05$). In contrast, IL-10 and CXCL10/IP-10 increased in GMSC-CM 100% and indirect co-culture conditions, indicating a shift toward an anti-inflammatory cytokine profile in vitro. Volcano plots (**Figure 2**) highlighted marked decreases in TNF-α (-4.9 log2FC, $P\_adj=0.002$) and IL-6 (-5.7 log2FC, $P\_adj=0.001$).

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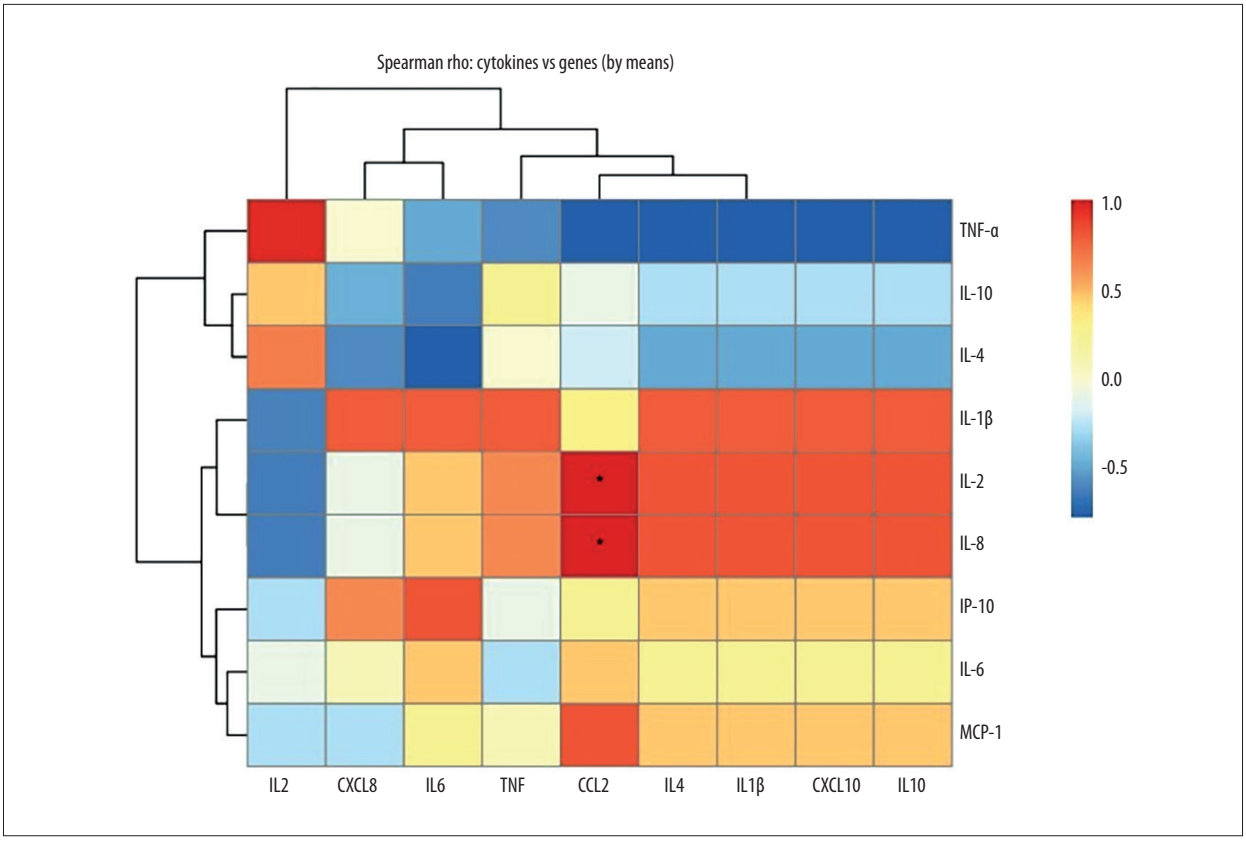

Figure 4. Correlation between the cytokines.

Gene Expression Pattern

RT-qPCR confirmed an increase in the levels of cytokines IL-10 and CXCL10 and decreased levels of IL6, TNF, and CCL2. Indirect GMSC co-culture and GMSC-CM 100% elicited the strongest responses depicted in **Figure 3**. Statistically significant fold changes were observed for IL6 (3.5 to 1.0), IL10 (1.0 to 3.0), and CXCL10 (1.0 to 3.1) (BH *P*_adj <0.05).

Correlation and PCA

Spearman correlation analysis, as seen in **Figure 4**, was used to describe concordance between selected transcripts and secreted cytokines across conditions; these associations are descriptive and do not establish mechanistic causality. PCA provided an unsupervised view of condition-level separation based on the combined cytokine/gene expression feature space, supporting a consistent shift in inflammatory signaling patterns with indirect GMSC exposure, as shown in **Figure 5**.

Discussion

Cancer development is linked to long-term inflammatory mediators, such as cytokines. Angiogenesis is encouraged in the cancer cells, and changes occur in the matrix proteins. HL PVL patients, those at different clinical phases of OSCC, and healthy individuals all showed that the multiplex cytokine test was effective in detecting and quantifying cytokine concentrations in their saliva. Currently, surgery, chemotherapy, radiation therapy, or a combination of these treatments are the most often used treatments for advanced OSCC. The 5-year overall survival rate for OSCC is still 50%, even with the utilization of numerous therapeutic modalities over the past 2 decades [23].

MSCs are a subset of mesoderm-derived non-hematopoietic stem cells that have multidirectional differentiation capability, rapid proliferation, and self-renewal. The blood vessel wall is one of the potential sources of MSCs, as it is a type of cell in the perivascular space. Its potential for tissue regeneration is thus quite high [24]. The use of MSCs in bone and nerve regeneration has been validated by numerous studies [25,26].

In reaction to inflammation and tissue damage, MSCs have chemotactic characteristics akin to those of immune cells. To control the inflammatory process and establish a balanced inflammatory and regenerative microenvironment in injured tissues, MSCs can release a variety of bioactive factors, including growth factors, chemokines, immunosuppressive molecules, and complement components, which can help treat a range

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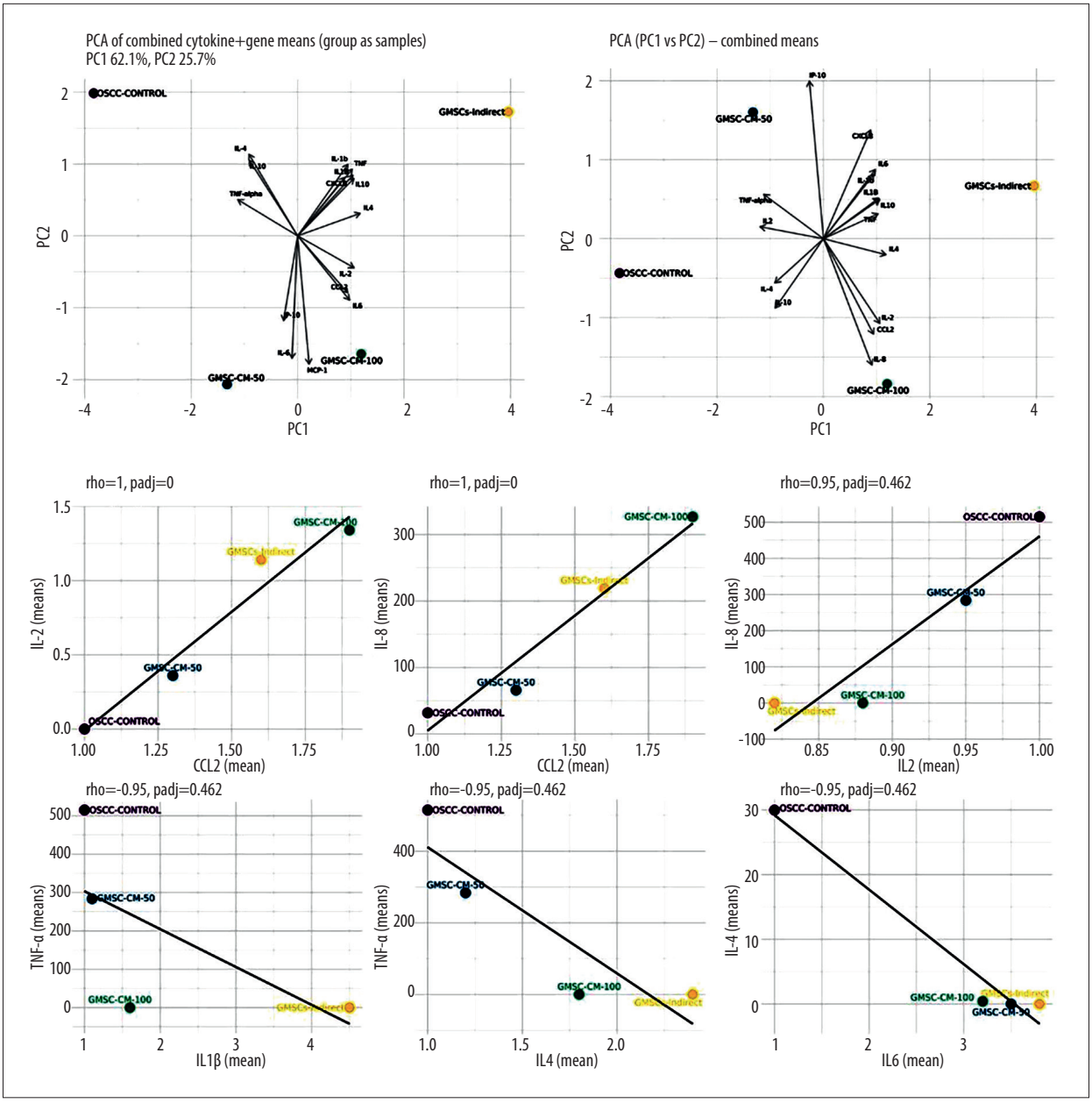

Figure 5. PCA.

of inflammatory and degenerative diseases [27]. Gingival-derived GMSCs are a promising source for stem cell-based therapy due to their facile isolation, quick growth, and strong immunomodulatory and anti-inflammatory capabilities [28]. By changing the microenvironment of surrounding oral cancer cells, GMSCs can stop the proliferation of oral cancer cells both in vitro and in vivo, suggesting that GMSCs can be used to treat mouth cancer and dysplasia [28].

A key factor in the metastatic cascade of OSCC is the EMT, a biological process in which epithelial cells lose their cell-cell adhesion qualities and acquire mesenchymal characteristics, hence gaining greater migratory and invasive capabilities. EMT facilitates distant metastasis in OSCC by allowing tumor cells to infiltrate surrounding tissue and reach the vasculature [29].

Because of its role in chronic inflammatory illnesses, the inflammatory mediator TNF-alpha NF-a) has been found to be associated with carcinogenesis. Low, continuous production can result in a tumor phenotype, which connects inflammation and carcinogenesis by acting as an endogenous tumor promoter. It can be used to forecast cancer patients' risk, responsiveness to treatment, and prognosis [30]. Although many studies have linked TNF levels with the histological differentiation

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of OSCC, few have correlated TNF levels with the degree of dysplasia [31].

Interleukin-6 (IL-6) can drive intercellular signal transmission and exchange by acting on cell receptors through either autocrine or paracrine pathways, thereby completing cell biological operations. The classical and trans-pathway transduction pathways are the 2 types of IL-6. In the traditional pathway, IL-6 can directly bind to IL-6R on the cell membrane to promote cell signal transduction. IL-6 plays a role in immune-mediated regulation, drug resistance, apoptosis, cancer formation, and differentiation. Patients with advanced tumors have also been reported to have noticeably increased levels of IL-6. IL-6, a target for diagnosis and treatment, is essential for the development of OSCC in terms of radio resistance, growth, and spread [32].

The recognition that IL-6 is a key cytokine promoting EMT marks a substantial breakthrough in our knowledge of how OSCC progresses. Elevated IL-6 levels were found in SCC25-GF CM by secretome analysis, and functional tests verified that IL-6 neutralization lessened the medium's ability to promote EMT. These results are in line with other studies that have shown IL-6 to be a strong EMT inducer in several malignancies, including breast and esophageal cancers [33].

Two biomarkers that we identified in this study—IL-10 and IL—8 showed a notable variation in expression levels between OSCC and control groups [34]. The interleukin-10 gene (IL10) functional polymorphism A1082G has been associated with a higher incidence of OSCC. According to earlier studies, patients with OSCC had higher levels of IL-10, suggesting that this pleiotropic cytokine may be crucial in the development of cancer. The gene expression of factors that influence inflammation is influenced by functional DNA polymorphisms, which may contribute to illness susceptibility, development, and severity. Few previous studies have documented higher serum/saliva IL-10 levels in patients with OSCC. Cytokines, particularly IL6, IL10, and TNF, are significant contributors to the incidence of OSCC [35]. To attain anticancer effects, GMSCs are also genetically altered to produce other cytokines, including IFN-γ, IL-2, IL-12, and IL-24 [36].

As a chemoattractant cytokine, IL-8, commonly referred to as a chemokine, is produced by a variety of blood and tissue cells. IL-8 is involved in angiogenesis, inflammation, and immune surveillance [37]. IL-8 acts as a potent angiogenic agent in tumors by controlling endothelial cell migration and proliferation. Previous studies have used a variety of methods to determine the role of IL-8 in OSCC tissue specimens, cell culture homogenates, and tissue lysates [38].

Numerous authors have discovered previously unidentified roles for GAPDH, many of which assume that the protein interacts with nucleic acids. Although some researchers have shown that GAPDH monomer or dimer can go to the nucleus in response to stress, the exact mechanism underlying this behavior is unknown. Some studies have shown that GAPDH has a role in DNA repair, either directly or indirectly [39].

Through paracrine signaling, our results indicate that indirect GMSC exposure is associated with attenuation of pro-inflammatory cytokine output from OSCC cells and relative increases in IL-10/CXCL10-associated cytokine signatures. Because the current model does not include immune cell components or functional tumor assays, these findings should be interpreted as changes in inflammatory cytokine signaling within OSCC cells in vitro.

The concordance between transcript-level and secreted cytokine measurements supports the internal consistency of our dataset; however, correlation and co-variation do not by themselves demonstrate direct regulatory interactions. Mechanistic inference would require pathway perturbation (eg, receptor blocking, knockdown/overexpression) and functional validation in immune-inclusive or 3D co-culture models.

A methodological limitation of the present study is that the tumor-derived primary cultures were not subjected to additional post-isolation molecular or immunophenotypic tumor-authentication assays. Therefore, the in vitro OSCC model should be interpreted as a low-passage epithelial-enriched primary culture derived from histopathologically confirmed malignant OSCC tissue, rather than as a fully authenticated long-term OSCC cell line. Nevertheless, the use of matched histopathologically confirmed tumor specimens, immediate primary culture establishment, epithelial-supportive keratinocyte serum-free medium after initial attachment, and exclusive use of passage 1 cells supported the relevance of the model for short-term paracrine signaling experiments.

Conclusions

Indirect signaling and GMSC-conditioned media reduced pro-inflammatory cytokine output from OSCC cells and increased IL-10 and CXCL10/IP-10-associated signatures, supporting a consistent pattern of altered inflammatory cytokine signaling in vitro. These data may motivate follow-up studies that incorporate functional tumor endpoints (eg, proliferation, migration/EMT markers) and immune cell components to determine whether these cytokine shifts translate into measurable changes in OSCC behavior and tumor-immune interactions.

Ethics Approval

This study was approved by the Research Ethics Committee at King Khalid University (HAPO-06-B-001) under Approval No. KKU-72-2025-21.

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Informed Consent

Written Informed consent was obtained from all the donors..

Data Availability

The data presented in this study are available on request from the corresponding author.

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Declaration of Figures' Authenticity

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