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SPATIAL ORGANIZATION OF THE TUMOR IMMUNE MICROENVIRONMENT IN ORAL SQUAMOUS CELL CARCINOMA: IMPLICATIONS FOR DIAGNOSIS, PROGNOSIS AND IMMUNOTHERAPY

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) develops inbetween a complex tumor immune microenvironment (TIME) which consists of lymphocytes, fibroblast, malignant cells, myeloid cells, endothelial cells & extracellular matrix. Increasing evidence suggest that the biological importance of these components depends not only on their abundance but also on their spatial distribution & interactions.

Objective: This narrative review summarizes the current evidence about the spatial organization of the TIME in OSCC & discusses its implications for prognosis, diagnosis & immunotherapy.

Methods: PubMed-indexed literature addressing the OSCC/HNSCC immune microenvironment, tumor-infiltrating lymphocytes, tertiary lymphoid structures (TLS), immune checkpoints therapy and spatial transcriptomics was narratively reviewed.

Results: Spatially distinct populations of CD8+ T cells, regulatory T cells, B cells, macrophages, myeloid-derived suppressor cells & cancer-associated fibroblasts contribute to immune activation or suppression. TLS, particularly their maturity and proximity to tumor cells, have emerged as important spatial determinants of prognosis and treatment response. Spatial transcriptomics and multiplex imaging have revealed metabolic, myeloid and stromal niches that are not apparent using conventional histopathology.

Conclusion: Spatial profiling may complement conventional clinicopathological & molecular biomarkers. Integration of immune-cell localization, , stromal organization, molecular signatures TLS architecture could improve risk stratification as well as facilitate more precise immunotherapy in OSCC.

KEYWORDS: oral squamous cell carcinoma; tumor immune microenvironment; spatial organization; spatial transcriptomics; tertiary lymphoid structures; tumor-infiltrating lymphocytes; immunotherapy; immune checkpoint inhibitors; PD-L1; cancer-associated fibroblasts.

1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the predominant malignant epithelial neoplasm of the oral cavity & remains a major global health problem. Despite advances in early detection & multimodal treatment, a considerable proportion of patients develop regional or distant disease, recurrence, or treatment resistance. The clinical behavior of OSCC is influenced by pathological characteristics and tumor stage. But it is also influence by complex interactions between malignant cells & the surrounding microenvironment.[1]

The tumor microenvironment (TME) contains immune cells, fibroblasts, endothelial cells, extracellular matrix and soluble mediators that can influence tumor growth, invasion, angiogenesis and immune surveillance. In head and neck squamous cell carcinoma (HNSCC), the immune landscape is particularly heterogeneous, with differences in

lymphocyte infiltration, immune-suppressive populations & immune checkpoint expression contributing to variation in clinical behavior & response to treatment.[2]

A significant limitation of conventional assessment is immune-cell abundance is often interpreted without considering its location. Tumor-infiltrating lymphocytes (TILs), for example, may be concentrated within tumor nests, restricted to the surrounding stroma, or distributed throughout both compartments. These arrangements can represent different biological states despite apparently similar overall lymphocyte densities. Intratumorally heterogeneity therefore represents an important determinant of tumor progression and therapeutic response.[3]

This has led to increasing interest in the spatial organization of the tumor immune microenvironment (TIME). Spatial analysis considers not only which immune cells are present but also where they are located, which cells are physically adjacent, and how immune and stromal compartments interact. This approach is particularly relevant to OSCC because the tumor can contain distinct metabolic, stromal and immune regions within the same lesion.

Recent advances in spatial transcriptomics have provided direct evidence of such heterogeneity in OSCC. Liu et al. demonstrated that different metabolic regions within OSCC were associated with distinct immune and stromal characteristics, including increased CD4+ T-cell infiltration and TGF-β expression in hypermetabolic regions. Their findings identified an epithelial cell–inflammatory cancer-associated fibroblast–regulatory T-cell axis that may contribute to local immune suppression.[4]

The spatial organization of myeloid cells also appears to change during OSCC progression. Recent single-cell and spatial-transcriptomic analysis showed that MDSCs undergo stage-dependent redistribution, while CD8+ T cells become spatially localized but functionally suppressed in advanced disease.[5] These observations suggest that immune suppression is not uniformly distributed throughout an OSCC but may arise within specific cellular neighborhoods.

Another important spatial component is the tertiary lymphoid structure (TLS). TLS are known to be the organized lymphoid aggregates that develop in chronically inflamed tissues & tumors. T-cell regions, B-cell follicles, antigen-presenting cells & specialized vasculature, are included in their organization. This potentially supports local antitumor immune responses.[6]

The increasing use of immune checkpoint inhibitors has also strengthened interest in the TIME. Pembrolizumab-based treatment have shown clinical benefit in recurrent or metastatic HNSCC, although the response remains variable between patients.[7] This variation cannot be completely explained by conventional biomarkers like PD-L1 expression. It highlights the need for additional biomarkers that reflect the functional state & spatial organization of the immune microenvironment.

The present narrative review examines the spatial organization of the TIME in OSCC, particularly emphasis on immune-cell distribution, myeloid and stromal niches, emerging spatial technologies, TLS, prognostic implications & potential applications in immunotherapy.

2. Spatial Organization of Immune Cells in OSCC

2.1 Tumor-infiltrating lymphocytes

TILs is one of the most extensively studied components of the immune microenvironment in OSCC. CD8+ cytotoxic T lymphocytes can recognize tumor-associated antigens & mediate destruction of malignant cells. CD4+ T-cell subsets can either support antitumor immunity or contribute to immune suppression.

A systematic review & meta-analysis showed that increased tumor infiltration by T lymphocytes, particularly CD3+ and CD8+ populations, is generally associated with more favorable outcomes in HNSCC.[8] But the prognostic value of TILs may depend on their spatial distribution.

Immune-cell penetration into the tumor compartment is a characteristic of immune-inflamed tumor. In contrast, an immune-excluded tumor contains immune cells chiefly within the surrounding stroma and with a limited penetration into the tumor nests. An immune-desert phenotype shows relatively sparse immune-cell infiltration. These patterns may have different biological consequences & may influence the effectiveness of immune checkpoint blockade.

Single-cell profiling has also shown the importance of specialized T-cell populations in head & neck cancer. Peng et al. demonstrated TCF1/TCF7-positive tumor-infiltrating T cells associated with TLS with a favorable prognosis.[9] These cells representing a population capable of sustaining antitumor T-cell responses. Their association with organized lymphoid structures emphasizes the biological relevance of T-cell population cannot always be understood alone from its density.

3. Myeloid Niches and Local Immune Suppression

Macrophages like myeloid cells as well as MDSCs, contribute substantially to the immunosuppressive TIME. These populations can inhibit T-cell activity, angiogenesis promotion alter cytokine networks & remodel the extracellular matrix.

Recent spatial analysis of OSCC has provided key evidence on MDSCs which occupies distinct anatomical regions during disease progression. Li et al. demonstrated that MDSCs were more closely associated with tumor regions in early disease but in advance cases it became concentrated at the tumor margin together with CD8+ T cells. This spatial redistribution accompanied by CD8+ T cells functional suppression.[5]

The same study also showed ANXA1-FPR2 signaling as a key mechanism of tumor–MDSC communication & showed in experimental models that interference with this pathway could enhance immune checkpoint blockade.[5] These outcomes provide an example of how spatially localized cellular interactions may contribute to therapeutic resistance.

The implication is important: a tumor may contain a considerable number of CD8+ T cells and still remain immunologically suppressed if these cells are positioned within a myeloid-rich, inhibitory niche. Consequently, future immune biomarkers may need to consider cellular neighborhoods rather than isolated cell populations.

4. Cancer-Associated Fibroblasts and Stromal Organization

Cancer-associated fibroblasts (CAFs) constitute an important non-malignant component of the OSCC TME. They produce extracellular matrix proteins, cytokines and chemokines and can influence tumor invasion, angiogenesis and immune-cell recruitment.

CAFs are heterogeneous and can adopt different functional states. Spatial transcriptomic analysis of OSCC has demonstrated that metabolic activity is associated with specific fibroblast phenotypes. In hypermetabolic regions, lactate generated by glycolytic epithelial cells was associated with transformation of fibroblasts into inflammatory CAFs. These cells expressed HIF1A and CXCL12, which promoted recruitment of regulatory T cells and increased TGF-β-associated immunosuppression.[4]

This finding illustrates the importance of considering metabolic and stromal features together with immune-cell distribution. The immune microenvironment is therefore not simply determined by the number of lymphocytes present; tumor metabolism and fibroblast organization can actively shape where immune cells accumulate and how they function.

5. Tertiary Lymphoid Structures: Organized Immune Niches

TLS is one of the clearest examples of organized immune architecture within tumors. Unlike diffuse lymphocytic aggregates, TLS display a degree of organization which resembles secondary lymphoid organs. Mature TLS may contain B-cell follicles with germinal-center features, antigen-presenting cells, T-cell zones & specialized high endothelial venules.

TLS can facilitate local antigen presentation, B-cell maturation & T-cell activation. It is also associated with favorable outcomes in several cancers.[6]

In OSCC, recent evidence specifically supports an association between TLS & prognosis. Ribeiro et al. reviewed six retrospective studies involving 1,203 patients & found that TLS were more commonly identified in the peritumoral compartment than within tumor tissue. Their presence was generally associated with improved survival, although substantial differences existed in TLS detection as well as in classification methods.[10]

Yao et al. subsequently evaluated TLS density, maturity and location in 189 OSCC patients. High TLS density was associated with improved overall survival and fewer lymph-node metastases. Importantly, the authors classified TLS into four groups according to maturity and location, and these spatial categories showed different associations with prognosis.[11]

These observations indicate that TLS should not be considered a simple binary biomarker. The biological significance of a TLS may depend on whether it is immature or mature, intratumoral or peritumoral, and whether it is located close to malignant cells.

6. TLS Maturity and Spatial Distribution

The importance of TLS maturity and spatial location has been demonstrated particularly clearly in recent HNSCC studies.

Xu et al. used digital spatial profiling and multiplex immunohistochemistry to characterize TLS at different stages of maturation and in different tumor compartments. Mature intratumoral TLS were enriched in memory B cells, plasma cells and CD4+ T cells and showed increased expression of genes related to B-cell receptor signaling and immune effector functions. In contrast, early TLS contained more endothelial cells, fibroblasts and regulatory T cells and demonstrated associations with epithelial–mesenchymal transition-related programs.[12]

Their clinical analysis showed that patients with mature intratumoral or invasive-margin TLS had better five-year survival, whereas mature peritumoral TLS were associated with less favorable outcomes.[12]

These observations are particularly relevant to OSCC because they demonstrate that the same type of immune structure may have different biological significance depending on its anatomical position.

One recent systematic review & meta-analysis by Morais et al., which included 17 studies involving more than 2,309 patients, further supports this concept. TLS-positive HNSCC was associated with improved overall and disease-free survival. The adverse effect of TLS absence persisted in the OSCC subgroup. The analysis also indicated that TLS maturity & spatial distribution influence prognosis.[13]

Therefore, future pathological assessment may need to distinguish TLS according to density, maturity and location rather than reporting only their presence or absence.

7. Spatial Technologies for Characterizing the TIME

Conventional histopathology and single-marker immunohistochemistry remain essential for diagnosis but provide limited information regarding complex cellular interactions.

Multiplex immunohistochemistry and multiplex immunofluorescence allow multiple cellular markers to be assessed simultaneously while preserving their spatial relationships. These approaches can identify immune-cell neighborhoods, tumor–immune interfaces and relationships between TLS and malignant cells.

Digital spatial profiling provides another level of analysis by measuring multiple proteins or transcripts within predefined tissue regions. More recently, spatial transcriptomics has enabled gene-expression profiles to be linked directly to tissue location.

The OSCC study by Liu et al. demonstrates the potential of spatial transcriptomics to identify metabolically distinct tumor regions and their associated immune and fibroblast populations.[4] Similarly, the study by Xu et al. used digital spatial profiling to characterize molecular differences between TLS of different maturation states and locations.[12]

Single-cell sequencing can identify transcriptionally distinct cellular states, whereas spatial transcriptomics determines where these states occur. Their combination therefore provides a more comprehensive representation of the TIME than either approach alone.

8. Implications for Diagnosis and Prognosis

Conventional OSCC prognostication relies heavily on tumor size, nodal involvement, depth of invasion, perineural invasion, extranodal extension and other pathological features. However, tumors with similar clinicopathological characteristics can show markedly different outcomes.

Spatial immune profiling may help explain some of this variation.

For example, high CD8+ T-cell density accompanied by close proximity to tumor cells may indicate active immune surveillance, whereas similar numbers of CD8+ cells restricted to stromal regions may indicate immune exclusion. Likewise, a mature intratumoral TLS may carry different prognostic information from an immature or distant lymphoid aggregate.

The OSCC TLS studies by Ribeiro et al. and Yao et al. support the prognostic importance of TLS density and location.[10],[11] The larger 2026 meta-analysis further indicates that TLS presence, maturity and spatial distribution may be useful prognostic features in HNSCC.[13]

Spatial biomarkers should therefore be considered complementary rather than alternative to conventional pathological staging. Their future clinical value will depend on reproducible scoring systems and validation in prospective cohorts.

9. Implications for Immunotherapy

Immune checkpoint blockade has changed the treatment landscape of recurrent and metastatic HNSCC. In the KEYNOTE-048 trial, pembrolizumab alone or combined with chemotherapy demonstrated clinically meaningful activity compared with cetuximab plus chemotherapy.[7]

Nevertheless, only a subset of patients achieves durable benefit. PD-L1 expression, particularly the combined positive score, is clinically useful but does not fully capture the complexity of the TIME.

Spatial immune architecture may provide additional predictive information. A tumor with PD-L1 expression but poor immune-cell penetration may differ biologically from a tumor in which PD-L1-positive cells coexist with abundant tumor-infiltrating CD8+ lymphocytes.

TLS are particularly promising in this context. Sadeghirad et al. analyzed recurrent/metastatic HNSCC using spatial proteomic, transcriptomic and single-cell approaches and found differences in TLS–tumor distance between patients who responded and those who did not respond to immune checkpoint inhibitors. Greater proximity of TLS to tumor cells was associated with treatment response.[14]

Ruiz-Torres et al. similarly investigated TLS spatial characteristics in patients receiving immune checkpoint blockade. Their analysis emphasized TLS proximity, composition and density as potential predictive features and demonstrated the limitations of relying exclusively on PD-L1 CPS.[15]

These findings suggest that future immunotherapy biomarkers may combine PD-L1 expression with spatial measurements of CD8+ T cells, B cells, myeloid cells and TLS.

10. Challenges and Future Directions

Despite the promise of spatial TIME analysis, several limitations must be addressed before these approaches can become routine clinical tools.

First, spatial technologies are technically demanding and may require expensive instrumentation, specialized tissue processing and advanced bioinformatics. Second, different studies currently use different definitions of TLS maturity, tumor proximity and immune-cell localization, making comparisons difficult.

Third, OSCC exhibits substantial intratumoral heterogeneity. A biopsy obtained from one region may not accurately represent the immune architecture of the entire tumor.[3] Spatial sampling therefore remains an important consideration when developing clinical biomarkers.

Future studies should establish standardized definitions and scoring systems for immune-cell density, immune exclusion, TLS maturity, TLS location and tumor–immune proximity. Prospective multicenter validation will be essential.

Longitudinal studies may also be particularly valuable because the TIME is dynamic. Surgery, radiotherapy, chemotherapy and immunotherapy can all modify immune-cell distribution and cellular interactions. Serial spatial profiling could therefore help identify mechanisms underlying treatment response and acquired resistance.

Artificial intelligence-assisted digital pathology may ultimately facilitate the translation of spatial biomarkers into routine diagnostic practice by automatically quantifying cell populations, distances, clustering patterns and cellular neighborhoods.

11. CONCLUSION

The TIME of OSCC is a highly organized and heterogeneous ecosystem in which the location and interaction of cells may be as important as their abundance. CD8+ T cells, regulatory T cells, MDSCs, macrophages, CAFs and B-cell-rich TLS occupy distinct spatial niches that influence immune surveillance, tumor progression and treatment response. Recent spatial-transcriptomic studies have demonstrated metabolic and myeloid heterogeneity within OSCC, while TLS studies have established the importance of density, maturity and anatomical distribution.[4,5,10–15]

The incorporation of spatial information into pathological assessment may therefore provide a more biologically meaningful characterization of OSCC than conventional cell-density measurements alone. In the future, combining spatial immune architecture with clinicopathological parameters, molecular alterations and established biomarkers such as PD-L1 may improve prognostic stratification and help identify patients most likely to benefit from immunotherapy.

Hence, spatial biomarkers remain an emerging field. Standardized definitions, prospective clinical validation & reproducible analytical methods are required before they can be incorporated into routine OSCC management.

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