Metabolic targeting of regulatory T cells in oral squamous cell carcinoma: new horizons in immunotherapy

Menglai Gan[1†], Nanshu Liu[2†], Wenting Li[1], Mingwei Chen[1], Zhongyu Bai[2], Dongjuan Liu[2*] and Sai Liu[1*]

Abstract

Oral squamous cell carcinoma (OSCC) is a prevalent oral malignancy, which poses significant health risks with a high mortality rate. Regulatory T cells (Tregs), characterized by their immunosuppressive capabilities, are intricately linked to OSCC progression and patient outcomes. The metabolic reprogramming of Tregs within the OSCC tumor microenvironment (TME) underpins their function, with key pathways such as the tryptophan-kynurenine-aryl hydrocarbon receptor, PI3K-Akt-mTOR and nucleotide metabolism significantly contributing to their suppressive activities. Targeting these metabolic pathways offers a novel therapeutic approach to reduce Treg-mediated immunosuppression and enhance anti-tumor responses. This review explores the metabolic dependencies and pathways that sustain Treg function in OSCC, highlighting key metabolic adaptations such as glycolysis, fatty acid oxidation, amino acid metabolism and PI3K-Akt-mTOR signaling pathway that enable Tregs to thrive in the challenging conditions of the TME. Additionally, the review discusses the influence of the oral microbiome on Treg metabolism and evaluates potential therapeutic strategies targeting these metabolic pathways. Despite the promising potential of these interventions, challenges such as selectivity, toxicity, tumor heterogeneity, and resistance mechanisms remain. The review concludes with perspectives on personalized medicine and integrative approaches, emphasizing the need for continued research to translate these findings into effective clinical applications for OSCC treatment.

Keywords Oral squamous cell carcinoma, Regulatory T cells, Metabolic targeting, Immunotherapy, Tumor microenvironment

†Menglai Gan and Nanshu Liu contributed equally to this work.

*Correspondence:
Dongjuan Liu
dongjuanliu@cmu.edu.cn
Sai Liu
liusai@cmu.edu.cn
[1] Department of Dental Materials, School and Hospital of Stomatology, China Medical University, Liaoning Provincial Key Laboratory of Oral Diseases, No. 117 Nanjing North Street, Heping District, Shenyang 110002, Liaoning, China
[2] Department of Emergency and Oral Medicine, School and Hospital of Stomatology, China Medical University, Liaoning Provincial Key Laboratory of Oral Diseases, No. 117 Nanjing North Street, Heping District, Shenyang 110002, Liaoning, China

Introduction

Oral squamous cell carcinoma (OSCC) is the most prevalent malignancy of the oral cavity, accounting for over 90% of oral cancers [1]. It represents a significant global health challenge, with higher incidences reported in regions like Southeast Asia and Europe, largely attributed to risk factors such as betel quid chewing, alcohol consumption, and tobacco use [2]. According to recent epidemiological estimates, OSCC contributes to approximately 389,000 new cases and 188,000 deaths annually worldwide [3]. Despite advancements in diagnostic and therapeutic strategies, the 5-year survival rate for OSCC remains at approximately 50%, largely due to late-stage diagnosis, frequent recurrences, and resistance to

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conventional therapies [4, 5]. Given its aggressive progression, substantial global burden, and the limitations of current therapies, there is an urgent need to develop more effective treatment strategies.

Regulatory T cells (Tregs), known for their immunosuppressive functions, play a pivotal role in the tumor microenvironment (TME) of OSCC (Fig. 1). While Tregs are essential for maintaining immune homeostasis, tumors often exploit these cells to evade immune surveillance [6]. In OSCC, Tregs suppress the the activity of various effector immune cells, including natural killer (NK) cells, dendritic cells (DCs), and B cells, simultaneously enhancing the immunosuppressive effects of cells such as tumor-associated neutrophils and myeloid-derived suppressor cells (MDSCs) [6–10]. This dual functionality makes Tregs a critical factor in the progression and immune evasion of OSCC.

Current research underscores the significant impact of Tregs on patient outcomes in OSCC, but several challenges remain. Studies have highlighted the importance of metabolic reprogramming in Tregs, which is essential for their function and survival within the TME. Key metabolic pathways, such as glycolysis, fatty acid oxidation, amino acid metabolism, and the PI3K-Akt-mTOR signaling pathway, enable Tregs to adapt to the harsh conditions of the TME, characterized by hypoxia and nutrient deprivation [11, 12]. Additionally, the oral microbiome plays a critical role in modulating Treg metabolism, adding complexity to the metabolic landscape of OSCC. Despite these insights, the precise mechanisms by which these metabolic pathways influence Treg function in OSCC remain poorly understood.

This review explores the metabolic dependencies of Tregs in OSCC and examines how targeting these pathways could pave the way for novel therapeutic strategies. By disrupting the metabolic support that sustains Treg function, it may be possible to enhance anti-tumor immunity and improve patient outcomes. However, the translation of these strategies into clinical practice is fraught with challenges. Key obstacles include
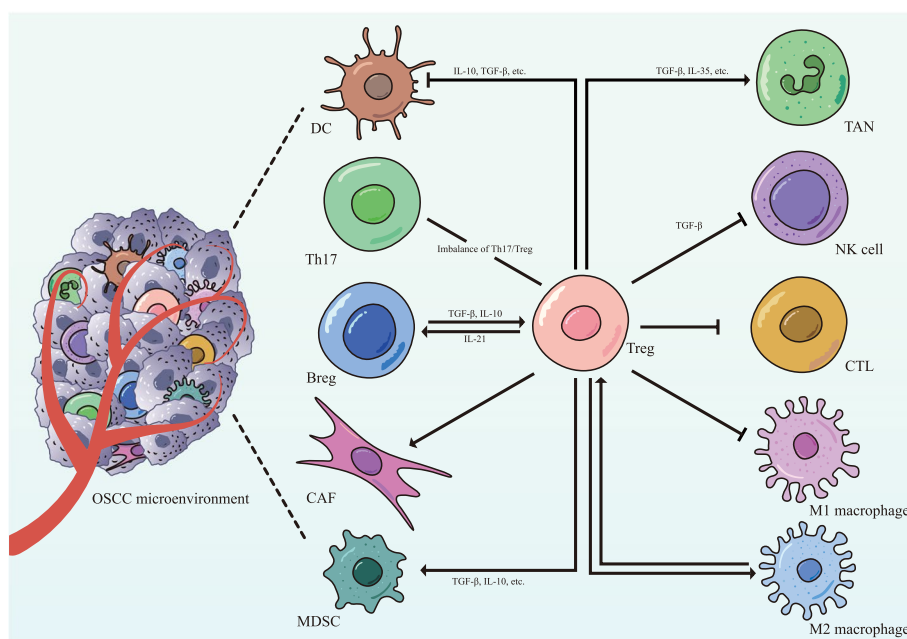

Fig. 1 Regulatory T cells as central modulators of immune dynamics in the tumor microenvironment of OSCC. The tumor microenvironment (TME) of oral squamous cell carcinoma (OSCC) represents a complex immunological landscape where regulatory T cells (Tregs) emerge as critical orchestrators of immunosuppression. Through multiple mechanisms, Tregs effectively suppress anti-tumor immune responses by inhibiting the cytotoxic functions of natural killer (NK) cells and CD8^{+} cytotoxic T lymphocytes (CTLs), while simultaneously suppressing the pro-inflammatory activities of M1 macrophages. By secreting immunosuppressive cytokines, primarily IL-10 and TGF-β, Tregs impair dendritic cell (DC) maturation and antigen presentation, promoting the development of tolerogenic DCs. Additionally, Tregs enhance the immunosuppressive functions of myeloid-derived suppressor cells (MDSCs) and tumor-associated neutrophils (TANs), thereby further inhibiting CTLs responses and contributing to tumor progression. This immunosuppressive network is reinforced by reciprocal interactions between Tregs, M2-polarized macrophages, and regulatory B cells (Bregs), creating self-sustaining circuits that foster tumor immune escape. Cancer-associated fibroblasts (CAFs) contribute by secreting chemokines that recruit Tregs and producing factors that stabilize their function. Additionally, the reduced Th17/Treg ratio observed in OSCC correlates with heightened immune tolerance and advanced disease progression. TME: tumor microenvironment; OSCC: oral squamous cell carcinoma; Treg: regulatory T cell; NK: natural killer; CTL: cytotoxic T lymphocyte; DC: dendritic cell; MDSC: myeloid-derived suppressor cell; TAN: tumor-associated neutrophils; Breg: regulatory B cell; CAF: cancer-associated fibroblast

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achieving selectivity in targeting Tregs without impairing other immune cells, managing tumor heterogeneity, and overcoming resistance mechanisms. The future of OSCC treatment may lie in integrative and personalized approaches. By combining metabolic targeting with other therapeutic modalities, it may be possible to develop more effective and tailored interventions, offering renewed hope for improved management of this challenging malignancy.

Metabolic adaptations of regulatory T cells in the TME of OSCC

Tregs play a crucial role in shaping the immunosuppressive environment of OSCC. Their metabolic programming is essential for maintaining their function and survival within the TME. Tregs exhibit significant metabolic flexibility that allows them to adapt to the harsh conditions of the TME in OSCC. Within the TME, Tregs navigate a range of metabolic stresses such as hypoxia, nutrient competition, and the immunosuppressive influence of immune checkpoints [13, 14].

Hypoxia and glycolytic shift in tregs

Hypoxia represents a fundamental characteristic of the TME in OSCC, emerging as a critical regulator of both tumor progression and immune cell function. This oxygen-deprived environment develops due to rapid tumor proliferation and abnormal vasculature. In response, Tregs in OSCC upregulate hypoxia-inducible factors (HIFs), particularly HIF-1α, to adapt to these oxygen-deprived conditions [15]. HIF-1α plays a crucial role in reprogramming Treg metabolism, driving a shift from oxidative phosphorylation to glycolysis [16–18]. This metabolic shift not only supports Treg survival and functionality under hypoxic conditions but also enhances their immunosuppressive capabilities. By prioritizing glycolytic, Tregs can maintain their regulatory functions within the hypoxic tumor niches, contributing to immune evasion and tumor progression.

Nutrient scarcity and alternative energy source

Within the TME, Tregs face severe nutrient scarcity due to competition from tumor cells and other immune cells for glucose, amino acids, and lipids. To adapt to these challenging conditions, Tregs exhibit metabolic flexibility that allows them to utilize alternative pathways, such as fatty acid oxidation (FAO) and amino acid metabolism, to meet their energy demands [19, 20]. This metabolic plasticity enables Tregs to maintain their suppressive functions even under conditions of glucose deprivation. Additionally, Tregs can metabolize lactate, a byproduct of aerobic glycolysis prevalent in the TME, to support their energy demands and maintain stability [21, 22].

Metabolic flexibility and adaptation in tregs

The ability of Tregs to switch between different metabolic pathways is a critical aspect of their adaptability in the TME [23, 24]. Beyond glycolysis and FAO, Tregs can utilize oxidative phosphorylation when conditions permit, thus exhibiting a high degree of metabolic flexibility [25, 26]. This adaptability is regulated by key signaling pathways such as the PI3K-Akt-mTOR axis, which integrates various metabolic cues and dynamically adjusts Treg metabolic programs. By fine-tuning their metabolic states, Tregs optimize their survival and immunosuppressive functions amidst the dynamic and hostile environment of the TME.

Regulation of treg metabolism by immune checkpoints.

Immune checkpoints, such as CTLA-4 and PD-1, play pivotal roles in modulating Treg function and metabolism within the TME [27]. Tregs express checkpoint molecules, including PD-1, CTLA-4, LAG-3, ICOS, and TIM-3, making them key targets for immune checkpoint blockade therapies. These checkpoints not only reinforce Treg-mediated immunosuppression but also influence their metabolic pathways. For instance, engagement of PD-1 on Tregs enhances FAO while suppressing glycolysis, promoting more sustainable energy production under nutrient-limited conditions [26, 28]. Additionally, the activation of these checkpoints helps Tregs conserve energy and maintain their suppressive phenotype despite the metabolic stresses of the TME. Specific checkpoints further shape Treg metabolic reprogramming. LAG-3 supports Treg immunosuppressive capabilities by modulating Myc-dependent pathways, while lactate absorption can upregulate PD-1 expression on Tregs [29, 30]. Similarly, glycolytic processes in Tregs are influenced by ICOS and TIM-3, with implications for their roles in tumor immunology and potential anti-tumor therapies [31, 32]. Targeting these immune checkpoints offers dual therapeutic benefits: disrupting Treg suppressive functions and altering their metabolic programming to reduce their immunosuppressive capacity in OSCC.

Core metabolic pathways driving regulatory T cell function in OSCC

Tregs rely on several key metabolic pathways to maintain their immunosuppressive functions and adapt to the harsh conditions of the TME. In OSCC, these pathways include glycolysis, fatty acid metabolism, amino acid metabolism, PI3K-Akt-mTOR pathway and the adenosine metabolism (Fig. 2).
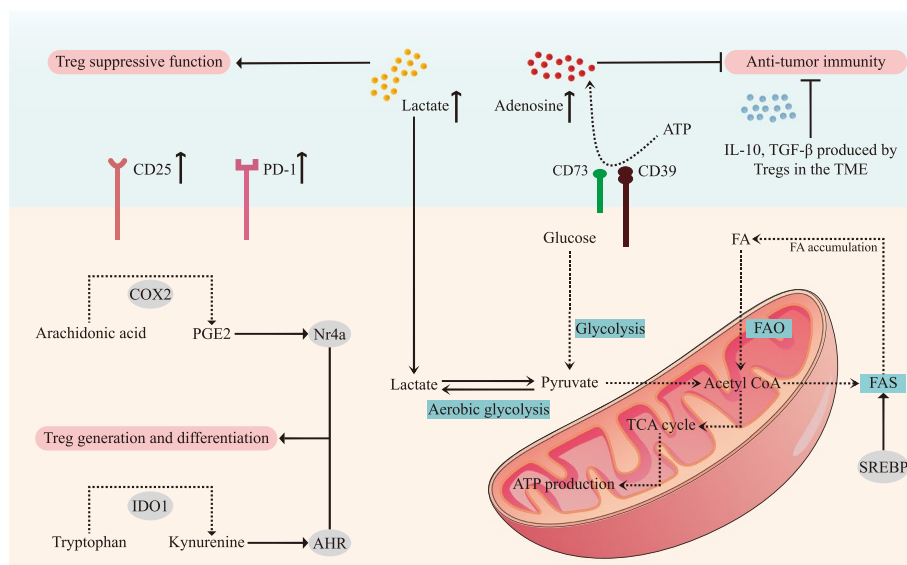
Fig. 2 Metabolic and immunosuppressive roles of Treg cells in the tumor microenvironment of OSCC. The tumor microenvironment (TME) of oral squamous cell carcinoma (OSCC) is characterized by elevated levels of immunosuppressive metabolites, with lactate accumulation from enhanced aerobic glycolysis and increased adenosine generation through CD39/CD73-mediated ATP hydrolysis, suppress the anti-tumor immune functions. To maintain their suppressive capabilities, Tregs undergo significant metabolic reprogramming, characterized by enhanced fatty acid oxidation (FAO) and fatty acid synthesis (FAS) pathways, regulated by sterol regulatory element-binding proteins (SREBPs). The metabolic interplay is further complicated by competition between Tregs and tumor cells for essential nutrients, including glucose and fatty acids, creating a metabolically hostile environment for anti-tumor immune responses. Critical molecular pathways, including the indoleamine 2,3-dioxygenase 1 (IDO1)-mediated conversion of tryptophan to kynurenine, activate the aryl hydrocarbon receptor (AHR) to promote Treg differentiation, while cyclooxygenase-2 (COX-2)-derived prostaglandin E2 (PGE2) enhances Treg accumulation through nuclear receptor 4a (Nr4a) upregulation. Tregs also secrete immunosuppressive cytokines, including IL-10, TGF-β, and IL-35, which suppress effector immune responses and facilitate tumor progression. Further, Tregs employ PD-1 and CD25 to maintain their suppressive phenotype and survival under the metabolically hostile conditions of the TME. TME: tumor microenvironment; OSCC: oral squamous cell carcinoma; FAO: fatty acid oxidation; FAS: fatty acid synthesis; SREBP: sterol regulatory element-binding protein; IDO1: Indoleamine 2, 3-dioxygenase 1; AHR: aryl hydrocarbon receptor; COX-2: cyclooxygenase-2; PGE2: prostaglandin E2; Nr4a: nuclear receptor 4a

Glycolysis and its impact on treg proliferation and suppression

Glycolysis is a fundamental metabolic pathway that provides rapid energy through the breakdown of glucose into pyruvate and ATP. This process is tightly linked to the function of various immune cells, supporting tumor growth, metastasis, and chemoresistance [33]. In the hypoxic and acidic TME of OSCC, where lactate accumulates, Tregs upregulate glycolytic enzymes to meet their energy demands [22, 34]. And a spatial transcriptomics study has revealed a connection between enhanced lactate utilization, Treg infiltration, and elevated HIF1A expression in OSCC [35]. The increased glycolytic activity not only facilitates Treg proliferation but also strengthens their immunosuppressive functions, enabling effective modulation of anti-tumor immune response. Moreover, glycolysis is positively correlated with the expression of PD-L1 in OSCC, which in turn contributes to immune evasion and tumor progression [36]. These findings suggest that the glycolytic pathway plays a central role in

Treg-mediated immunosuppression within the OSCC TME.

Fatty acid oxidation and the long-term survival of tregs in OSCC

FAO is another critical metabolic pathway for Tregs, providing sustained energy through the breakdown of fatty acids. FAO is particularly important in nutrient-deprived environments like the TME of OSCC. FAO provides sustained energy and essential reducing equivalents, supporting the long-term survival and stability of Tregs [25, 37–39]. Inhibition of mTOR, a key regulator of metabolism, shifts Tregs from glycolysis to FAO, further reinforcing their immunosuppressive functions [40]. High expression of the fatty acid receptor CD36 in OSCC has been linked to metastasis and mitochondrial health in Tregs, enabling adaptation to lactate-rich environments [41, 42]. CD36 might support mitochondrial health and biogenesis of Treg by providing lipid signals, and program Tregs to adapt to lactate-rich TME (Fig. 3D) [24]. Arachidonic acid (AA) metabolism, a polyunsaturated
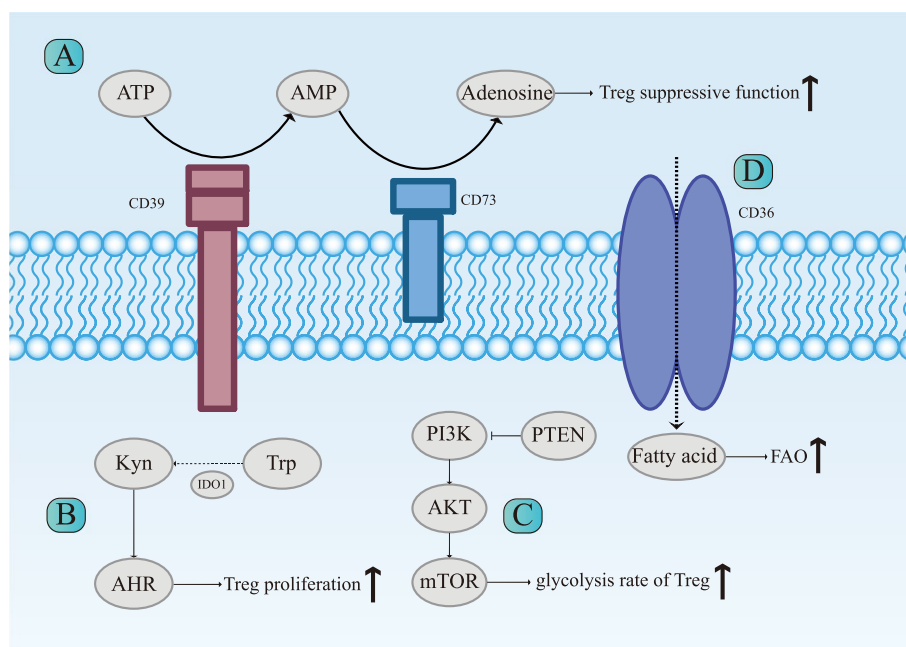
Fig. 3 Key metabolic pathways modulating Tregs functions in the tumor microenvironment of OSCC. The suppressive function of regulatory T cells (Tregs) in the tumor microenvironment (TME) of oral squamous cell carcinoma (OSCC) is shaped by key metabolic pathways, including the CD39/CD73 adenosine axis (**A**), tryptophan (Trp)–kynurenine (Kyn)–aryl hydrocarbon receptor (AHR) pathway (**B**) and phosphatidylinositol 3-kinase (PI3K)-protein kinase B (Akt)-mammalian target of rapamycin (mTOR) Pathway (**C**). The CD39/CD73 cascade hydrolyzes ATP to adenosine, which enhances Treg suppressive functions and inhibits effector T cells. The Trp–Kyn–AHR pathway, mediated by indoleamine 2,3-dioxygenase 1 (IDO1), converts Trp to Kyn, activating AHR to promote Treg stability and immunosuppression. PI3K-Akt-mTOR signaling supports glucose uptake, glycolysis, and lipid metabolism to adapt to the nutrient-deprived TME. **D** Additionally, CD36 is upregulated in tumor-infiltrating Tregs, enhancing fatty acid uptake and fatty acid oxidation (FAO). This adaptation allows Tregs to survive and function effectively in the lactate-rich and nutrient-deprived TME. Treg: regulatory T cell; TME: tumor microenvironment; OSCC: oral squamous cell carcinoma; Trp: tryptophan; Kyn: kynurenines; AHR: aryl hydrocarbon receptor; PI3K: phosphoinositide 3-kinases; AKT: protein kinase B; mTOR: mammalian target of rapamycin; PTEN: phosphate and tensin homologue; IDO1: indoleamine 2, 3-dioxygenase 1; FAO: fatty acid oxidation

fatty acid, were significantly activated in OSCC patients. Cyclooxygenase-2 (COX-2), an enzyme that plays a crucial role in AA metabolism, has also been observed with an increase in OSCC [43]. Prostaglandin E2 (PGE2), a COX-2 product, promotes tumor progression by increasing Treg numbers through inducing nuclear receptor 4a (Nr4a) expression [44, 45]. FAO thus enables Tregs to maintain their suppressive activities even when glucose availability is limited, thus ensuring their persistence in the TME.

Amino acid metabolism: the kynurenine pathway and its role in treg-mediated immune evasion

Amino acid metabolism, particularly tryptophan metabolism via the kynurenine pathway, plays a significant role in Treg function in OSCC [46–48]. The enzyme indoleamine 2,3-dioxygenase 1 (IDO1) converts tryptophan to kynurenine, which activates the aryl hydrocarbon receptor (AHR), enhancing Treg immunosuppressive capabilities (Fig. 3B) [49–51]. IDO has been reported to be a significant negative prognostic factor in patients

with OSCC, contributing to immune evasion by triggering MDSC activation and proliferation through Tregs [7, 52]. Other amino acids, such as arginine and glutamine, also support Treg function and proliferation [53–55]. The manipulation of amino acid metabolism offers a potential strategy to disrupt Treg-mediated immune suppression in OSCC.

PI3K-Akt-mTOR pathway: central to treg metabolic control and immune suppression

The PI3K-Akt-mTOR signaling pathway is a crucial regulator of Treg metabolism, integrating various metabolic signals to control cell growth, proliferation, and survival [29, 56]. This pathway is also involved in the cell proliferation as well as glucose, fatty acid and amino acid metabolism [57, 58]. In OSCC, this pathway modulates glucose uptake, glycolysis, and lipid metabolism, ensuring that Tregs can adapt to the dynamic conditions of the TME (Fig. 3C) [54, 59, 60]. Activation of the PI3K-Akt-mTOR pathway supports Treg survival and enhances their suppressive function

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by regulating key metabolic enzymes and transporters [26, 61, 62]. Moreover, this pathway influences the expression of immune checkpoints such as CTLA-4 and PD-1, further promoting the immunosuppressive environment conducive to tumor growth [63].

The pro-tumor mechanisms of PI3K-Akt-mTOR pathway include mutations of the phosphate and tensin homologue (PTEN), which is an upstream inhibitor of PI3K-Akt-mTOR signaling and plays a role in maintaining Treg cell stability [64–66]. Specifically, the targeted elimination of PTEN not only impairs Treg mitochondrial health and upregulates glycolysis but also reduces the expression of CD25 and FOXP3, collectively undermining Treg homeostasis and functional capacity [66, 67].

Nucleotide metabolism and its role in treg function

Nucleotides are the fundamental building blocks of genetic material, playing a role in metabolic biosynthesis and serving as one of forms of energy utilization within the body [68]. Tumor cells exhibit enhanced nucleotide diversity and active nucleotide biosynthesis. In OSCC, the expression of purine derivatives (e.g., hypoxanthine) and nucleosides (e.g., guanosine) are upregulated, contributing to tumor progression [69]. Targeting nucleotide metabolism, including nucleic acid biosynthesis substrates and energy sources within the TME, has the potential to enhance anti-tumor immune responses. The metabolism of folate, a key pathway for nucleotide synthesis, supports T cell activation, proliferation, and survival [70]. The expression of folate-metabolism associated methylenetetrahydrofolate dehydrogenase (MTHFD) family genes has been identified as potential biomarkers for Treg infiltration in OSCC [71].

ATP, a purine analogue, plays a pivotal role in modulating immune responses by activating immune cell receptors. In OSCC, ATP is converted to adenosine through the enzymatic actions of CD39 and CD73, especially when Tregs undergo apoptosis. The biological effects of extracellular adenosine are mediated by G-protein–coupled adenosine receptors, which are classified into A1, A2A, A2B, and A3 subtypes (Fig. 3A) [72]. Overexpression of CD73 in the OSCC microenvironment results in elevated adenosine levels, which are associated with poor patient prognosis [73]. Adenosine exerts immunosuppressive effects by reducing neutrophil adhesion and promoting macrophage production. Exogenous adenosine impairs the migratory ability of conventional T cells while enhancing Treg expansion and immunoregulatory activity via A2AR engagement [74, 75].

The pentose phosphate pathway: a critical metabolic hub in tregs

The pentose phosphate pathway (PPP) and the glycolytic pathway are two interconnected metabolic routes involved in glucose uptake and utilization. The PPP supplies essential precursors for nucleotide biosynthesis and generates NADPH, which is critical for fatty acid synthesis and redox balance. Notably, the PPP is frequently upregulated in tumor cells. Inhibitors targeting key enzymes of the PPP, such as glucose-6-phosphate dehydrogenase (G6PD), have been shown to suppress the proliferation and metastasis of OSCC in vivo [76, 77]. PPP is also regulated by the mTOR pathway, with activation of mTORC1 stimulating PPP activity [78]. Moreover, the PPP has been implicated in T cell metabolism and is thought to influence the Th17/Treg balance, potentially impacting immune regulation within the TME [79].

Influence of the oral microbiome on treg metabolism in OSCC

The oral microbiome, consisting of bacteria, fungi, and viruses, profoundly shapes the metabolic and immune landscape of Tregs within the TME of OSCC [80, 81]. Increasing attention has also been directed toward outer membrane vesicles (OMVs), spherical double-layered structures released by Gram-negative bacteria during growth or in response to environmental stress. With diameters ranging from 50 to 250 nm, OMVs carry diverse biomolecules, including proteins, lipopolysaccharides (LPS), phospholipids, and periplasmic components, playing a pivotal role in regulating the TME [82]. This section explores the interplay between the oral microbiome and Treg metabolism, emphasizing the composition of the microbiome, microbial metabolites and immune modulation.

Microbial composition and its role in OSCC development

The oral microbiome exhibits significant compositional alterations in OSCC patients compared to healthy individuals, characterized by increased microbial diversity and dysbiosis [83]. Certain microorganisms, such as *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Candida albicans*, human papillomavirus (HPV) and Epstein-Barr virus (EBV) are more prevalent in OSCC and have been implicated in tumor progression [84–91]. These microorganisms contribute to OSCC progression through various mechanisms. For example, *Candida* induces metabolic abnormalities within tumors, causing mitochondrial damage and promoting the progression of OSCC [92, 93]. Recent studies also highlight the role of OMVs from oral bacteria in influencing OSCC behavior. OMVs derived from *Fusobacterium nucleatum* have been

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shown to activate autophagy and disrupt protein homeostasis in OSCC cells, driving metastasis [94]. Similarly, *Porphyromonas gingivalis* OMVs promote tumor invasion and migration [95]. Interestingly, some researches show a possible antitumorigenic effect of *Aggregatibacter actinomycetemcomitans*-derived OMVs in OSCC, highlighting an area for further research [96].

Microbial metabolites influencing treg metabolism

Microbial metabolites significantly impact Treg function and metabolism. Elevated level of lactate and other metabolites in the TME enhance the stability and suppressive function of Tregs [97, 98]. For instance, *Candida albicans* enhances metabolite production in OSCC cells, potentially influencing Treg activity indirectly [99]. EBV infection reprograms cellular metabolism, favoring the Warburg effect, which increases lactate production and supports Treg function [100]. Additionally, LPS, a major OMV component, mediates inflammation and drives Treg accumulation in OSCC, contributing to the suppression of anti-tumor immunity [101]. These findings reveal a metabolic link between microbial metabolites and Treg activity, further underscoring the complex interplay between the microbiome and TME.

Immune modulation by the oral microbiome in OSCC

The oral microbiome exerts profound effects on immune modulation in OSCC, particularly by influencing Treg activity [80, 102, 103]. Microbial dysbiosis disrupts immune homeostasis, promoting an environment that favors Treg-mediated immunosuppression [104, 105]. HPV infection is associated with metabolic reprogramming in tumor cells, including increased glycolysis, which supports Treg activity in head and neck squamous cell carcinoma (HNSCC) [106, 107]. HPV also enhances mTOR activity under hypoxic conditions and promotes HIF-1 accumulation, leading to metabolic dysregulation in OSCC. While HPV does not directly correlate with overall survival (OS) in OSCC patients, HPV-positive patients often have better prognoses, potentially due to microbiome-mediated immune modulation [108, 109].

Within the OSCC TME, bacteria such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum* activate key signaling pathways, including PI3K/Akt and CDH1/β-catenin, promoting tumor proliferation and survival [110, 111]. The microbiome also contributes to tumor cell invasion, migration, and Treg differentiation and amplification through the secretion of specific metabolites. *Fusobacterium nucleatum* influences purine metabolism, generating metabolites that support Treg survival and function within the TME [112–114]. Additionally, it drives OSCC progression by enhancing glycolysis through GLUT1-mediated lactate production,

creating an immunosuppressive environment that favors Treg activity [22, 115, 116]. Similarly, *Porphyromonas gingivalis* affects ATP metabolism in tumors and converts ethanol into acetaldehyde, a carcinogenic intermediate that further accelerates OSCC progression [117]. Moreover, outer membrane vesicles (OMVs), known to modulate microbial metabolism, are being investigated as a novel delivery system for cancer vaccines. Notably, OMVs have been shown to reduce Treg populations in tumor-draining lymph nodes, highlighting their potential to regulate Tregs and reshape the tumor immune microenvironment [118].

Targeting treg metabolism as a therapeutic strategy in OSCC

The metabolic targeting of Tregs in OSCC offers promising therapeutic potential by disrupting the metabolic support of Tregs, thereby reducing their suppressive function and enhancing anti-tumor immunity (Table 1). These strategies, which include the inhibition of glycolysis, modulation of fatty acid metabolism and amino acid metabolism, PI3K-Akt-mTOR pathway inhibitors, and adenosine metabolism as well as oral microbiome therapy are actively being explored in clinical trials, showing significant potential for improving patient outcomes in OSCC.

Inhibition of glycolysis

Tregs in the TME of OSCC rely heavily on glycolysis for rapid energy production, particularly under hypoxic conditions. Inhibitors of glycolytic enzymes, such as 2-deoxy-D-glucose (2-DG), impair Treg function by limiting their primary energy source [148, 149]. Recent studies have demonstrated the synergistic effects of combining glycolysis inhibition with chemotherapy or radiotherapy, enhancing anti-tumor efficacy by inducing apoptosis, DNA damage, and metabolic reprogramming [150]. Importantly, glycolysis inhibitors selectively target Tregs without significantly affecting effector T cells, which can utilize alternative metabolic pathways. Ongoing clinical trials aim to evaluate the potential of these inhibitors in mitigating Treg-mediated immunosuppression to improve cancer therapy outcomes.

Modulating fatty acid metabolism

FAO is crucial for the long-term survival and suppressive function of Tregs in nutrient-deprived environments like the TME [28]. FAO inhibitors, such as etomoxir, disrupt Treg metabolism by blocking the oxidation of fatty acids, thereby reducing their suppressive capacity [40]. Targeting CD36-mediated lipid metabolism using selective inhibitors has shown potential for enhancing anti-tumor immunity in OSCC [151]. This approach takes advantage

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Table 1 Clinical Trials Targeting Metabolism Pathways Related to Head and Neck Carcinoma

Strategies	Key Molecules/Targets	Inhibitors/Drugs	NCT Number	Main Results
Inhibition of Glycolysis	Glycolysis/ Glucose analog	2-DG	NCT00096707	11 pts (32%) had SD, 1 pts (3%) PR and 22 pts (66%) PD as best response RD of 2DG with weekly docetaxel 63 mg/kg/day [119]
	Anaerobic glycolysis	Dichloroacetate	NCT01386632	No significant differences in Gr 3/4 AE rates between dichloroacetate and placebo CR rates were higher in the dichloroacetate group [120]
Modulating Fatty Acid Metabolism	CD36	ABT-510	NCT00113334	2 pts had SD, 1 pt had PD 6/6 pts affected by AEs [121, 122]
	3-hydroxy-3-methyl glutaryl coenzyme A reductase	Atorvastatin	NCT04915183	No results posted
			NCT02022293	Enrollment target could not be achieved
Inhibition of Amino Acid Metabolism	IDO	Epacadostat	NCT02318277	Phase 2 ORR 12.0%, higher in CPI-naive (16.1%) vs. previous CPI (4.1%) [123]
			NCT02178722	84% pts (n=52) experienced TRAEs, Gr 3/4 TRAEs reported in 24% pts, and 100 mg Epacadostat twice per day plus 200 mg pembrolizumab every 3 weeks recommended for phase II evaluation [124]
			NCT02327078	For pts on Epacadostat 300 mg, preliminary DCR was 70%, and 48% pts (n=42) reported TRAEs [125, 126]
		BMS-986205	NCT03854032	Higher pTE in radiographic responders vs. non-responders (85% vs. 5%), and higher pTE in responders in the lymph node compartment (73% vs. 23%), while CR demonstrated in 6/16 (38%) pts [127, 128]
Targeting Nucleotide Metabolism	PARP	Olaparib	NCT02882308	Olaparib treatment modulates DNA damage response network and exerts extra antitumor effect by elevating oxidative stress in HNSCC patients [129]
	A2aR	NIR178	NCT03207867	For HNSCC pts with NIR178 160 mg twice daily plus PDR001 with no immuno-oncology therapy ORR 13.3 [130]
Modulating Glucose and Fatty Acid Metabolism	PI3K	Buparlisib (BKM120)	NCT02113878	1 of 7 pts experienced Gr 4 rash on DL1, DLTs observed (4 of 6 pts) on DL2, and 5 pts respond to buparlisib monotherapy [131]
			NCT01816984	Gr3 AEs reported in 10 pts, and no DLTs during dose escalation, while PR (n=1) and SD (n=4) reported [132]
		Copanlisib	NCT02822482	2 of 3 pts experienced DLT on 45 mg DL, and no DLT was reported on 30 mg DL. Median PFS 2.66 months, and median OS 6.01 months [133]
		Alpelisib (BYL719)	NCT02537223	No DLT observed at 200 mg, 2 of 2 pts reported DLTs at 250 mg, and RP2D declared at 200 mg. 3-year PFS and OS rate both 77.8% [134]
			NCT01602315	1 PR, 3 unconfirmed PRs and 5 SDs at 300 mg, and 1 PD and 1 SD at 400 mg. RP2D declared as 300 mg QD (whole tablets) [135]
		Bimiralisib	NCT03740100	ORR 17%, median PFS 5 months and median OS 7 months. 62.5% pts (n=5) experienced 14 severe AEs [136, 137]
	mTOR	Sirolimus(rapamycin)	NCT01195922	1 pt had a pathologic CR, while 25% pts (n=4) reported response (1 CR, 3 PRs, 12 SDs) [138]
		Temsirolimus (TORISEL)	NCT01016769	OR rate 43% (n=13) with 1 CR, 10 confirmed PRs, and 2 unconfirmed PRs. OS 12.9 months [139]

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Table 1 (continued)

Strategies	Key Molecules/Targets	Inhibitors/Drugs	NCT Number	Main Results
			NCT01172769	Median PFS 56 days, median OS 152 days, and PFR 40% at 12 weeks. 87.5% pts (n = 35) experienced at least two AEs [140]
		Everolimus (RAD001)	NCT01333085	No DLT in phase I, and everolimus RD declared at 50 mg/w, while 2.6% pts experienced a CR (n = 1), 76.3% PR (n = 28), and 21% SD (n = 8). OR rate 79% [141]
			NCT01051791	CBR 28%, median PFS 1.5 months, and median OS 4.5 months. 2 SDs observed (5.5 and 4.5 months) [142]
		CC-115	NCT01353625	22% pts reported Gr 3 drug-related AEs. SD observed 53% [143]
	EGFR	Cetuximab	NCT01252628	Median PFS 80 days, no CR found, and 7 PR confirmed. Median OS 211 days vs. 256 days (cetuximab plus PX-866 vs. cetuximab alone) [144]
			NCT01256385	Median PFS both 3.5 months. (cetuximab plus Temsirolimus vs. Temsirolimus alone) Median OS 177 days vs. 176 days and OR rate 12.5% vs. 2.5% [145]
		Duligotuzumab	NCT01577173	PFS 4.2 months vs. 4.0 months (duligotuzumab vs. cetuximab), OS 7.2 months vs. 8.7 months, and OR rate 12% vs. 14.5% [146]
		Erlotinib	NCT00942734	OR rate 2.8% at 12 weeks, median PFS 11.9 weeks and median OS 10.25 months 8% pts (n = 3) achieved PR at 4 weeks [147]
Targeting Oral Microbiome	Oral microbiome	N-Acetyl Cysteine	NCT03982537	Concept is withdrawn and a different concept will be submitted the near future
	Oral microbiome	Probiotic Lozenge (ProDentis Lozenge)	NCT04925700	No results posted
	Oral microbiome	MET-4	NCT03838601	No results posted

Abbreviations: *ORR* Objective response rate, *PI3K* Phosphoinositide 3-kinase, *CPI* Immune checkpoint inhibitor, *pts* Patients, *Gr* Grade, *Trp* Tryptophan, *Kyn* Kynurenine, *DCR* Disease control rate, *TRAE* Treatment-related adverse event, *pTE* Pathologic treatment effect, *CR* Complete response, *HNSCC* Head and neck squamous cell carcinoma, *PFR* Progression-free survival rate, *DL* Dose level, *DLT* Dose limiting toxicity, *AE* Adverse event, *PR* Partial response, *SD* Stable disease, *CI* Confidence interval, *PFS* Progression free survival, *OS* Overall survival, *RP2D* Recommended phase 2 dose, *PD* Progressive disease, *RD* Recommended dose, *OR* Overall response, *CBR* Clinical benefit rate

of the reliance of Tregs on FAO, making them more vulnerable to metabolic intervention. Although research on Treg-specific modulation of fatty acid metabolism remains limited, combining FAO inhibitors with immune checkpoint inhibitors may further amplify anti-tumor immune responses by weakening Treg-mediated immunosuppression.

Modulating amino acid metabolism
Amino acid metabolism, particularly the tryptophan-kynurenine- AhR pathway, is another target for disrupting Treg function [51, 152, 153]. Inhibitors of indoleamine 2,3-dioxygenase (IDO), such as epacadostat, reduce the production of kynurenine, thereby attenuating Treg proliferation and suppressive activity [154, 155]. By blocking this pathway, IDO inhibitors can decrease Treg-mediated immunosuppression and enhance the efficacy of other cancer therapies. Clinical trials are currently evaluating the effectiveness of IDO inhibitors in combination with immune checkpoint inhibitors in OSCC and other cancers, highlighting their potential to improve therapeutic outcomes.

PI3K-Akt-mTOR pathway inhibitors
The PI3K-Akt-mTOR signaling pathway is a critical regulator of Treg metabolism, controlling processes such as glucose uptake, glycolysis, and lipid metabolism. Persistent activation of the PI3K-Akt-mTOR signaling route is common in OSCC [156]. Inhibitors of this pathway, such as rapamycin, impair Treg survival and function by disrupting these metabolic processes [12]. These inhibitors reduce Treg-mediated immunosuppression and promote anti-tumor immunity. In OSCC, epidermal growth factor receptor (EGFR) overexpression, an upstream activator

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of the PI3K pathway, contributes to tumor progression [156]. It is noticed that EGFR-targeted antibody, such as cetuximab, increased the frequency of CD4+FOXP3+ intratumoral Treg in HNSCC, demonstrated that combining parsaclisib (a PI3K inhibitor) with itacitinib (a JAK1 inhibitor) significantly reduced Treg populations and increased the CD8/FoxP3 ratio in solid tumors [63, 157]. Additionally, trials with ipatasertib (an AKT inhibitor) in head and neck cancers aim to improve the TME by reducing hypoxia, enhancing radiotherapy efficacy [158]. These findings support the use of PI3K-Akt-mTOR inhibitors in combination therapies to improve OSCC treatment outcomes.

Targeting nucleotide metabolism

Research indicates that Foxp3 expression in Tregs is closely associated with nucleotide metabolism, glycolysis, and lipid metabolism, highlighting the metabolic flexibility of Tregs in adapting to different pathways [26]. Nucleotides, as essential components of DNA and RNA, also serve as key regulators of intracellular signaling pathways. Therapeutic strategies targeting nucleotide metabolism hold significant promise for OSCC treatment. CD39 and CD73 are essential to adenosine metabolism, which affect Treg activity. Preclinical studies and mouse tumor models have demonstrated the efficacy of agents that block CD39 and CD73 activity or employ selective A2AR inhibitors to disrupt adenosine-mediated immunosuppression [159, 160]. These inhibitors, including CD39/CD73 blockers and A2a/A2b receptor antagonist, effectively reduce the immunosuppressive capacity of Tregs mediated by adenosine [161–163]. Notably, adenosine metabolism is enhanced in OSCC, further contributing to tumor progression [164]. Although clinical trials specifically targeting nucleotide metabolism in Tregs remain limited, ongoing research underscores the potential of adenosine metabolism inhibitors to reduce tumor growth and enhance anti-tumor immune responses. Further investigations are needed to elucidate the molecular mechanisms underlying these therapies to facilitate their clinical application.

Targeting oral microbiome

The oral microbiome's influence on Treg metabolism offers exciting opportunities for therapeutic interventions in OSCC. Targeting specific microbial populations or their metabolites can reshape the metabolic environment of the TME, reducing Treg-mediated immunosuppression and enhancing anti-tumor immunity. For instance, manipulating the abundance of *Fusobacterium nucleatum* or *Prevotella intermedia* has been shown to alter the TME, improving the efficacy of immunotherapies [165–168]. Remarkably, a combined treatment involving

Fusobacterium nucleatum OMVs and oncolytic viruses significantly decreased Treg populations and upregulated PD-L1 expression in tumor tissues, thereby enhancing the effects of immune checkpoint therapies [169]. Moreover, probiotics or antibiotics could be explored as potential therapies to restore a healthy microbiome balance, thereby modulating Treg function and enhancing anti-tumor immune responses. Such approaches underscore the importance of the oral microbiome as a novel and promising target in OSCC treatment.

Challenges and future directions for targeting regulatory T cell metabolism in OSCC

Tregs in OSCC holds significant promise for improving therapeutic outcomes. However, the translation of these strategies from research to clinical application faces several challenges. Addressing these challenges while exploring future perspectives is crucial for advancing this therapeutic approach.

Selectivity and toxicity of treg metabolic inhibitors

One of the primary challenges in targeting Treg metabolism is achieving selectivity without causing toxicity to other immune cells. Many metabolic pathways that are essential for Tregs function also play crucial roles in effector T cells and other immune cells [170–172]. For instance, glycolysis and FAO are not exclusive to Tregs, and inhibiting these pathways could impair the broader immune response, potentially leading to unintended immunosuppression or other adverse effects [28, 173]. To overcome this, therapies must be designed to selectively target Tregs or be administered in a way that minimizes impact on non-target cells. Research on Treg-specific depletion has focused on targets such as CD25, CCR4, and CTLA-4, but these strategies have thus far shown limited efficacy [174]. Given that Tregs selectively rely on certain metabolic pathways, combining metabolic targets with Treg-specific approaches could help balance efficacy and toxicity in future therapies.

Tumor heterogeneity: a barrier to uniform therapeutic responses

The heterogeneity of the TME in OSCC poses a significant challenge for uniform therapeutic responses to metabolic targeting. Variations in oxygen levels, nutrient availability, and metabolic adaptations among different tumor regions can influence how Tregs and other cells respond to metabolic inhibitors [175–177]. OSCC is categorized into various subtypes, including basal, mesenchymal, classical, and atypical, each with distinct gene characteristics, protein expression, and clinical features. For example, the atypical subtype is closely associated with HPV infection, which further highlights the role of

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the microbiome in OSCC progression [178]. These variabilities necessitate a more personalized approach to therapy, considering the unique metabolic landscape of each tumor. Identifying biomarkers to reflect these metabolic differences could enable more tailor treatments, improving efficacy and minimizing side effects.

Resistance mechanisms to metabolic inhibitors in tregs

Tregs, like cancer cells, can develop resistance mechanisms to metabolic inhibitors [179]. In OSCC, Tregs may adapt to metabolic inhibition through mechanisms such as upregulation of alternative metabolic pathways, altered expression of metabolic enzymes, or changes in cell damage and apoptosis [8, 180–184]. For instance, Foxp3 reprograms Treg metabolism by suppressing Myc, promoting lipid metabolism over glucose metabolism [25]. Tregs also exhibit metabolic changes in mitochondrial function and lipid metabolism through fatty acid binding proteins (FABPs) mediated pathways [185]. Furthermore, Treg apoptosis can promote immunosuppressive resistance in the TME by contributing to adenosine production, which in turn shields tumor cells from anti-tumor therapies. Understanding these resistance mechanisms is critical for developing combination therapies that can prevent or overcome resistance. Continuous monitoring and adaptive treatment strategies may be required to manage resistance and sustain therapeutic efficacy. Future research should focus on elucidating these resistance mechanisms and developing combination therapies that prevent or counteract resistance. This could involve targeting multiple metabolic pathways simultaneously or using adaptive treatment strategies to stay ahead of tumor and Treg adaptations.

Managing immune-related adverse events in treg targeting therapies

Manipulating the metabolism of immune cells can lead to immune-related adverse events (irAEs), which can range from mild to severe [186, 187]. These irAEs arise because the metabolic pathways targeted in Tregs are also present in other immune cells, leading to unintended activation or suppression. Careful monitoring and management of irAEs are essential to ensure patient safety during treatment. Strategies such as dose adjustment, combining therapies with immunosuppressive drugs, and close clinical monitoring are necessary to mitigate these risks.

Personalized medicine and integrative approaches for metabolic targeting

Personalizing metabolic targeting strategies based on the unique metabolic profile of each patient's tumor could significantly improve treatment outcomes [188]. Advances in genomics, metabolomics, and proteomics offer the potential to identify biomarkers that predict response to specific metabolic inhibitors. Tailoring treatments to individual patients' metabolic landscapes will enhance efficacy while minimizing off-target effects, leading to more precise and effective therapies.

Combining metabolic inhibitors with existing cancer treatments, such as immune checkpoint inhibitors, chemotherapy, and radiation therapy, may further enhance therapeutic efficacy [189]. For example, CD36 antagonists have been shown to deplete Tregs by affecting lipid metabolism, which restores the therapeutic effect of PD-1 antibodies in certain tumors [190]. In addition, the interaction between ICOS and the mTOR pathway could also affect Treg metabolism, offering another potential avenue for combination strategies [31]. These integrative approaches aim to synergize the effects of different modalities, potentially overcoming the limitations of monotherapies. Preclinical studies and clinical trials should focus on identifying optimal combinations and treatment schedules to maximize anti-tumor responses while minimizing adverse effects.

Exploring novel metabolic targets in tregs and the role of the oral microbiome in shaping treg metabolism for future therapies

Exploring new metabolic targets and pathways specific to Tregs within the OSCC microenvironment is crucial for developing innovative therapies. Beyond well-known pathways like glycolysis and FAO, future research should investigate less explored metabolic pathways, such as the pentose phosphate pathway, with a focus on tumor-immune interactions. These studies could offer critical insights into Treg metabolism in tumor progression and immune evasion. High-throughput screening techniques and CRISPR-based genetic screens can aid in the discovery of novel metabolic vulnerabilities in Tregs [191].

The oral microbiome plays a significant role in modulating Treg metabolism and function. Therapeutic strategies aimed at manipulating the microbiome—using probiotics, prebiotics, or antibiotics—could influence Treg activity and enhance anti-tumor immunity [192, 193]. Further research is needed to unravel the complex interactions between the microbiome and Treg metabolism, potentially leading to microbiome-based adjunct therapies for OSCC.

Transformative potential of emerging technologies of metabolism research

Tumor metabolism and immunometabolism have become increasingly important fields for understanding tumorigenesis, progression, and therapeutic resistance. While previous research primarily focused on conventional cellular experiments and in vivo animal studies, advancements in multi-omics, single-cell sequencing, and spatial techniques have provided new opportunities for exploring metabolic dynamics in both tumor and immune microenvironments [194]. Multi-omics technologies allow the quantification of metabolites in the TME, revealing the metabolic profiles of tumor and immune cells [195]. Single-cell technologies facilitate the identification of distinct cellular populations with varying metabolic states, providing insight into Treg metabolic changes. Spatial techniques further elucidate metabolic activities across tumor tissue regions, which can help identify the metabolic preferences of tumor-infiltrating Tregs [196]. Combining these emerging technologies will provide a comprehensive understanding of the TME and offer robust support for the translational application of metabolism-targeted immunotherapy.

Conclusions

The metabolic targeting of Tregs in OSCC has emerged as a promising strategy to enhance anti-tumor immunity. This review has provided a comprehensive overview of the metabolic targeting of Tregs in OSCC. It highlights critical metabolic pathways, such as glycolysis, FAO, amino acid metabolism, nucleotide metabolism, and the PI3K-Akt-mTOR pathway, which are essential for maintaining Treg function under the nutrient- and oxygen-deprived conditions of the TME. Additionally, we have highlighted the emerging role of the oral microbiome as a crucial modulator of Treg metabolism and OSCC pathogenesis.

Therapeutic strategies targeting these metabolic dependencies show significant potential in disrupting Treg-mediated immunosuppression. These approaches include glycolysis inhibitors, FAO modulators, amino acid metabolism blockers, and innovative treatments leveraging insights from OMVs. However, the heterogeneous nature of OSCC necessitates personalized therapeutic approaches that consider tumor-specific metabolic signatures, spatial metabolic variations, and individual patient characteristics. The advancement of this field relies heavily on cutting-edge technologies, including multi-omics platforms, single-cell sequencing, spatial metabolomics, and advanced molecular imaging techniques. These tools are essential for developing effective personalized metabolic targeting strategies and understanding the complex interactions within the TME. Future research should focus on developing novel metabolic targeting approaches, understanding resistance mechanisms, optimizing combination therapies, and successfully translating preclinical findings into clinical applications.

The successful implementation of these therapeutic strategies requires a coordinated effort among researchers, clinicians, and regulatory bodies, along with rigorous clinical validation and continuous technological innovation. While challenges remain in addressing the complexity of the TME and the dynamic nature of metabolic processes, metabolic targeting of Tregs represents a potentially transformative approach in OSCC treatment. Through continued research and collaborative efforts, this emerging therapeutic paradigm offers new hope for patients battling this aggressive malignancy, potentially revolutionizing the treatment landscape of OSCC.

Abbreviations

OSCC	Oral squamous cell carcinoma
Treg	Regulatory T cell
TME	Tumor microenvironment
NK	Natural killer
DC	Dendritic cell
MDSC	Myeloid-derived suppressor cell
HIF	Hypoxia-inducible factor
FAO	Fatty acid oxidation
AA	Arachidonic acid
COX-2	Cyclooxygenase-2
PGE2	Prostaglandin E2
Nr4a	Nuclear receptor 4a
IDO	Indoleamine 2, 3-dioxygenase
AHR	Aryl hydrocarbon receptor
PTEN	Phosphate and tensin homologue
MTHFD	Methylenetetrahydrofolate dehydrogenase
PPP	Pentose phosphate pathway
G6PD	Glucose-6-phosphate dehydrogenase
OMV	Outer membrane vesicle
LPS	Lipopolysaccharides
HPV	Human papillomavirus
EBV	Epstein-Barr virus
EV	Extracellular vesicle
HNSCC	Head and neck squamous cell carcinoma
OS	Overall survival
2-DG	2-Deoxy-D-glucose
EGFR	Epidermal growth factor receptors
FABPs	Fatty acid binding proteins
irAEs	Immune-related adverse events

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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