Research Progress on the Regulation of Immune Microenvironment by Internal and External Factors in Oral Squamous Cell Carcinoma (OSCC)

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Abstract. Oral squamous cell carcinoma (OSCC) is one of the most prevalent malignant tumors globally, and its prognosis is severely constrained by chemotherapy resistance. Existing treatment methods often face bottlenecks due to selective toxicity and recurrent resistance. In recent years, combination chemotherapy and targeted therapy have become important strategies to overcome resistance by regulating the tumor immune microenvironment (TME). Research has revealed multidimensional resistance mechanisms, including abnormal signaling pathways, non-coding RNA regulation, and TME remodeling. This article systematically analyzes the intrinsic and extrinsic regulatory factors of cisplatin resistance in OSCC: On the intrinsic level, CMTM6 activates the Wnt pathway through the ENO-1/AKT/GSK3β axis, HPRT1 upregulates the MMP1/PI3K/Akt pathway, and the imbalance of the GAS5/miR-196a axis drives resistance; on the extrinsic level, circ-ILF2 induces M2 polarization of macrophages and the secretion of pro-drug resistance factors by CAFs, thereby remodeling the TME. The study suggests that targeting PD-L1, the PI3K/AKT pathway, or combining immune checkpoint inhibitors can significantly reverse resistance. These results provide a molecular basis for optimizing OSCC treatment strategies, with molecules such as CMTM6 and circ-ILF2 serving as potential biomarkers or therapeutic targets. Future research should focus on large-scale clinical trials to validate the efficacy of combination therapies, integrate single-cell sequencing with spatial omics to analyze dynamic changes in the TME, and explore emerging mechanisms such as metabolic reprogramming to promote personalized treatment and improve patient outcomes.

Keywords: oral squamous cell carcinoma; resistance; chemotherapy; immunotherapy; targeted therapy.

1. Introduction

Oral cancer is a malignant tumor that occurs in the oral cavity and is the sixth most common cancer worldwide, with an estimated 177,757 cancer-related deaths in 2020. More than 90% of oral cancer cases belong to the histological category of OSCC [1]. The current standard treatments for OSCC include surgery, chemotherapy, and radiotherapy [2]. Surgery remains the most effective treatment for OSCC, but it inevitably damages the function and aesthetics of the oral and facial regions. Furthermore, they have difficulties in dealing with metastatic tumors in conjunction with radiotherapy. Chemotherapy can inhibit rapidly growing cells, including those at metastatic sites, by suppressing cell growth and division. However, its selective toxicity is relatively low, and normal cells with high proliferation rates (such as hair follicles, bone marrow, and the gastrointestinal tract) may also be harmed. To reduce toxicity to normal cells, chemotherapy drugs are usually administered at suboptimal doses, but this can lead to eventual treatment failure and even drug resistance and metastatic disease.

Therefore, people have consistently been keen on developing targeted anti-cancer drugs to increase selective toxicity in cancer treatment [3].

Due to the rapid growth pattern of OSCC and the high risk of metastasis and recurrence, its prognosis remains poor. Chemotherapy can partially improve the prognosis of OSCC patients, but chemoresistance is a major obstacle to successful treatment. Therefore, it is crucial to explore the underlying mechanisms of chemotherapy resistance in OSCC and find solutions to overcome it [4].

Single chemotherapy is difficult to break through resistance, and combination targeted therapy is needed to reshape TME.

Targeted therapies work by altering specific signaling pathways in cancer cells or by targeting the delivery of anticancer drugs to the cancer region, increasing the dose of drugs reaching the malignant tissue, and avoiding adverse side effects on normal tissue by targeting specific receptors. To date, the FDA has approved two molecularly targeted therapies for the treatment of OSCC. The first is an EGFR-targeted therapy, the Epidermal Growth Factor Receptor (EGFR), which is a member of the ErbB family of receptor tyrosine kinases, and the other recently approved OSCC-targeted therapy is an anti-PD1 therapy based on immune checkpoint blockade.3 Cisplatin (CDDP) is a commonly used chemotherapeutic agent in the treatment of OSCC.

The aim of this paper is to summarize the strategy of chemotherapy combined with targeted therapy to modulate TME to overcome CDDP resistance through two aspects: intrinsic factors (tumor cells' own signaling abnormalities, e.g., molecularly mediated resistance such as Circ-ILF2, CMTOR6, etc.) and extrinsic factors (TME inhibition, e.g., M2 macrophage, Tregs increase) to break through drug resistance and enhance the efficacy of chemotherapy.

2. Cancer Stem Cells and Epithelial-mesenchymal Transition

Cancer stem cells (CSCs) and epithelial-mesenchymal transition (EMT) are key mechanisms driving chemoresistance in head and neck cancer. EMT promotes tumor resistance to chemotherapeutic agents like cisplatin by endowing cells with migratory and invasive capabilities as well as stem cell-like properties. In nasopharyngeal carcinoma (NPC), NEDD4 protein enhances cisplatin resistance by maintaining the EMT phenotype, while the upregulation of miR-139-5p can reverse EMT and promote chemosensitivity.

In head and neck squamous cell carcinoma (HNSCC), the activation of the IL-6/STAT3 pathway induces a mixed EMT phenotype, which is associated with cisplatin resistance mediated by Aldo-Keto Reductase Family 1 Member C1 (AKR1C1). In OSCC, drug-resistant cells are often accompanied by high expression of CSC markers such as CD44, Oct-4, aldehyde dehydrogenase-1 (ALDH1), and enhanced self-renewal capacity. For example, hyaluronic acid (HA) interacts with Oct-4-Sox2-Nanog through CD44v3 to activate miR-302, upregulating the survival proteins cIAP-1, cIAP-2, and XIAP, thereby promoting the stemness and cisplatin resistance of CSCs. Furthermore, the inhibition of aldehyde dehydrogenase 1 family member A1 (ALDH1A1) or FGF2 signaling can weaken CSC characteristics and restore chemotherapy sensitivity. Long non-coding RNA FOXD2-AS1 maintains cancer stemness by activating STAT3 and is associated with chemoresistance and poor prognosis in laryngeal squamous cell carcinoma. In summary, targeting the EMT-CSC regulatory network (such as NEDD4, STAT3, ALDH1, and FGF2) may provide new strategies to overcome drug resistance in head and neck cancer [5].

3. DNA Damage

Cisplatin triggers the formation of phosphorylated histone H2AX (γH2AX) at DNA damage sites by activating the ATR-CHEK1/2 signaling pathway, and the abnormal DNA damage response (DDR) signaling in HNSCC is closely related to cisplatin resistance.

Research has found that in HNSCC cells treated with cisplatin, the functional depletion of DDR effector proteins (such as WDHD1, DSCC1, etc.) inhibits ATR phosphorylation and weakens γH2AX foci formation, suggesting that impaired DDR signaling is a key mechanism of cisplatin resistance.

Clinical analysis shows that high expression of ERCC1 (a DNA repair gene) in biopsy tissues before treatment in HNSCC patients can predict poor response to 5-FU/cisplatin chemotherapy, further validating the regulatory role of DNA repair capacity on chemotherapy sensitivity.

Targeted therapeutic strategies for the DDR pathway are being explored in clinical trials: ATR inhibitors (such as M6620, ceralasertib) in combination with cisplatin/radiotherapy (NCT02567422,

NCT02264678), aiming to enhance the efficacy of chemotherapy by blocking ATR-mediated checkpoint activation.

In addition, the phase I/II trial of the PARP inhibitor veliparib combined with carboplatin-paclitaxel confirmed its safety and synergistic anti-tumor potential. The phase I trial of the WEE1 inhibitor AZD1775 combined with cisplatin/docetaxel also showed good tolerance, with 5 out of 9 patients with stage III/IVB HNSCC achieving partial remission and 4 maintaining stable disease.

These studies highlight that targeting key DDR nodes (ATR, PARP, WEE1) can synergize with chemotherapy to overcome HNSCC resistance, providing new directions for optimizing treatment [5].

4. Epigenetic Modification

Cisplatin-based chemoresistance in head and neck squamous cell carcinoma (HNSCC) can be regulated by epigenetic mechanisms. High methylation of the CpG island in the promoter of the neurofilament light polypeptide (NEFL) gene inhibits its expression, leading to the downregulation of tuberous sclerosis complex 1 (TSC1), which in turn activates the mTOR pathway, promoting cisplatin resistance. Clinical analysis shows that high methylation of NEFL is associated with shortened patient survival, while neoadjuvant treatment with rapamycin (an mTOR inhibitor) can inhibit tumor growth, with significant clinical remission observed in 4 out of 16 HNSCC patients. Additionally, the histone-modifying protein PAK2 inhibits glycolysis and enhances chemotherapy resistance by upregulating the c-Myc/PKM2 axis, and its high expression is associated with poor prognosis in HNSCC.

Non-coding RNAs play a crucial role in regulating drug resistance: miR-629-3p promotes cisplatin resistance by enriching drug metabolism and EMT-related genes, and its expression is associated with reduced patient survival; miR-96-5p drives radioresistance and cisplatin resistance in p53 mutant HNSCC cells by targeting PTEN (inhibiting the PI3K-AKT pathway). Currently, a clinical trial (NCT03953443) is exploring the predictive value of microRNA and epigenetic silencing on the treatment response of HPV-negative HNSCC. These findings suggest that targeting epigenetic regulatory nodes (such as mTOR, PAK2, and miRNA) or combining them with traditional chemotherapy may provide new strategies for reversing HNSCC resistance [5].

4.1. Escape of Cell Apoptosis

Chemoresistance in head and neck squamous cell carcinoma (HNSCC) is closely associated with an imbalance of apoptosis-regulating proteins. Survivin (BIRC5), an anti-apoptotic protein, is significantly overexpressed in HNSCC tumors and HPV-negative patients, and mutations in its gene (e.g., p.Phe93Ser) diminish its anti-apoptotic function, thereby enhancing radiotherapy sensitivity. The small molecule inhibitor YM155 reverses cisplatin resistance and inhibits tumor proliferation in in vitro and in vivo models by reducing survivin levels, suggesting the potential to target BIRC5. In addition, high expression of XIAP (E3 ubiquitin ligase) correlates with poor cisplatin efficacy. siRNA inhibition of XIAP or the use of the dual antagonist ASTX660 in combination with radiotherapy significantly enhances chemo-sensitivity and delays the growth of HPV+/HPV- xenografts. Resistance to TRAIL (apoptosis-inducing ligand) in HPV-positive HNSCC cells was lifted by bortezomib (proteasome inhibitor) by a mechanism involving caspase activation, upregulation of TRAIL-R2 expression, and G2/M phase blockade, while XIAP depletion synergistically enhanced TRAIL efficacy. These findings suggest that targeting anti-apoptotic proteins (e.g., BIRC5, XIAP) or combining apoptotic pathway activators (e.g., TRAIL, bortezomib) may overcome chemoresistance in HNSCC by modulating apoptotic thresholds, providing a molecular basis for the development of combination therapy strategies [5].

4.2. Tumor Microenvironment（TME）

Chemotherapy resistance in head and neck squamous cell carcinoma (HNSCC) is closely related to the tumor hypoxic microenvironment (TME) and angiogenesis regulation. Hypoxia promotes

angiogenesis by activating the ALK1-BMP9/10 signaling pathway, while ALK1-Fc (ligand trap) combined with cisplatin can significantly inhibit tumor growth in HNSCC models, reduce hypoxia, and improve vascular stability. Early clinical trials have shown its anti-tumor potential. The SDF-1/CXCR4 axis participates in the progression of HNSCC by regulating matrix metalloproteinases and angiogenesis. Its high expression is associated with cisplatin chemotherapy resistance, but the specific mechanisms still need to be elucidated. Targeting angiogenesis (such as pazopanib and bevacizumab) combined with chemotherapy has shown partial efficacy in clinical trials, but further optimization is needed. Tumor-associated fibroblasts (CAFs) are key components of the TME, inducing cisplatin resistance in HNSCC by secreting growth factors (such as midkine, a heparin-binding growth factor that promotes carcinogenesis and chemoresistance) and exosomal miR-196a (targeting CDKN1B/ING5) and enhancing migration, invasion, and proliferation. Clinical studies have shown that the enrichment of CAFs and tumor-associated macrophages (TAMs) is associated with poor prognosis and resistance to 5-fluorouracil-based radio-chemotherapy in OSCC patients. In addition, the plasma level of CAF-derived exosomal miR-196a can serve as a potential biomarker for chemoresistance. Targeting CAF-mediated signaling pathways (such as miR-196a and midkine) or combining anti-angiogenesis/immunotherapy (such as atezolizumab (humanized IgG1 antibody against PD-L1) + bevacizumab (monoclonal antibody developed against VEGF), NCT03818061) may provide new strategies for reversing HNSCC resistance [5].

4.3. Internal and External Factors

4.3.1 CMTM6 Drives Cisplatin Resistance by Regulating Wnt Signalling through the ENO-1/AKT/GSK3β Axis

In chemotherapy-resistant OSCC, CMTM6 has been identified as a key resistance driver. Global proteomic analysis shows that CMTM6 is significantly overexpressed in cisplatin-resistant cell lines and in OSCC patients who do not respond to chemotherapy, and it is associated with poor recurrence-free survival in patients with esophageal cancer, head and neck cancer, and lung squamous cell carcinoma. Functional studies indicate that knocking down CMTM6 can restore the sensitivity of resistant cells to cisplatin, while overexpression reverses this effect. In vivo experiments confirmed that cisplatin-induced cell death is enhanced and tumor burden is reduced in CMTM6-depleted OSCC xenograft tumors. Mechanistically, CMTM6 stabilizes the expression of membrane-bound enolase-1 through interaction, activating the AKT/GSK-3β-mediated Wnt signaling pathway to promote cell survival. Additionally, CMTM6 acts as a PD-L1 stabilizer, simultaneously mediating tumor immune evasion and chemoresistance. These findings reveal a new mechanism by which CMTM6 drives resistance through the regulation of the Wnt pathway and immune checkpoints, suggesting that targeting CMTM6 or combining it with immunotherapy (such as anti-PD-L1) may provide new strategies to overcome cisplatin resistance in OSCC [6].

4.3.2 HPRT1 Promotes Chemoresistance in OSCC through Activation of the MMP1/PI3K/Akt Signalling Pathway

Hypoxanthine-guanine phosphoribosyl transferase 1 (HPRT1) has traditionally been regarded as a housekeeping gene, but studies have found its significantly high expression in head and neck squamous cell carcinoma (HNSCC), particularly in OSCC, and its close association with poor prognosis and cisplatin (CDDP) resistance in patients. Clinical analysis shows that HPRT1 expression in OSCC tumor tissues is significantly higher than in adjacent non-tumor tissues, and patients with high expression have a shorter survival period. Functional experiments confirm that knocking down HPRT1 can enhance the sensitivity of OSCC cells to cisplatin, while overexpression exacerbates resistance; in animal models, HPRT1 inhibition significantly reduces tumor burden. Mechanistically, RNA sequencing revealed that HPRT1 drives resistance by upregulating matrix metalloproteinase MMP1 and activating the PI3K/AKT pathway, with its expression levels positively correlated with MMP1 and phosphorylated AKT. These findings suggest that HPRT1 is not only a potential biomarker for poor prognosis in OSCC but also mediates chemotherapy resistance by regulating the

MMP1/PI3K/AKT axis. This provides a new strategy for targeting HPRT1 or combining pathway inhibitors (such as PI3K/AKT inhibitors) to reverse resistance [7].

4.3.3 GAS5 Attenuates Cisplatin Resistance in OSCC Via Sponge miR-196a

Long non-coding RNA GAS5 plays a key regulatory role in cisplatin (DDP) resistance in OSCC. The study found that GAS5 is lowly expressed in DDP-resistant OSCC cell lines and patient tissues, and its expression level is positively correlated with patient survival rates. Functional experiments showed that overexpression of GAS5 can significantly restore the sensitivity of resistant cells (CAL27/DDP) to DDP and promote apoptosis. Mechanistically, GAS5 inhibits the expression of miR-196a by directly targeting it (confirmed by luciferase reporter and RNA immunoprecipitation assays), and exogenous miR-196a transfection can reverse the chemoresensitization effect mediated by GAS5. Moreover, GAS5 exhibits a cytoplasmic distribution in OSCC tissues, and its low expression is associated with the DDP-resistant phenotype. These results indicate that the GAS5/miR-196a axis is an important pathway in OSCC chemotherapy resistance. Targeting this axis (e.g., by restoring GAS5 expression or inhibiting miR-196a) may provide new strategies for reversing cisplatin resistance and offer potential biomarkers for prognosis evaluation and personalized treatment [8].

4.3.4 Upregulation of a Cytokine (Circ-ILF2) in OSCC Promotes Cisplatin Resistance and Induces Macrophage M2 Polarisation

Circular RNA circ-ILF2 has been revealed as a key regulatory factor in cisplatin (CDDP) resistance in OSCC. The study found that circ-ILF2 is significantly overexpressed in CDDP-resistant OSCC cell lines, and its circular structure stability was verified by RNase R and actinomycin D. Functional assays confirmed that circ-ILF2 overexpression enhances cell survival and inhibits apoptosis, thereby exacerbating CDDP resistance, while knockdown reverses the resistant phenotype. Mechanistically, circ-ILF2 acts as a molecular sponge for miR-1252, competitively binding and inhibiting miR-1252 activity, thereby relieving the transcriptional repression of the downstream target KLF8 (Krüppel-like factor 8) by miR-1252, leading to the upregulation of KLF8 protein expression and driving resistance. In addition, circ-ILF2 also induces tumor-associated macrophages (TAM) to polarize towards the pro-tumor M2 phenotype, suggesting that it synergistically promotes chemotherapy resistance by remodeling the immune microenvironment. This study first elucidates the role of the circ-ILF2/miR-1252/KLF8 axis in cisplatin resistance in OSCC and reveals its new function in regulating TAM polarization, providing a potential strategy for targeting circ-ILF2 or combined immunotherapy (such as blocking M2 polarization) to reverse resistance [2].

4.3.5 Tolypothrix Dichloromethane Ethylacetate Fraction（TDEF）Inhibits Cisplatin-resistant H357 Cells Via PI3K/AKT/β-catenin Pathway

A study explored the roles of TDEF and H357cisR in the treatment of OSCC, revealing that TDEF enhances the activity of p53, BAX, and MMP, while H357cisR inhibits caspase-3/7 and β-catenin, preventing cell death and promoting cell survival [9].

CDDP-induced up-regulation of p62 protein, facilitated by the EGFR-PI3K-Akt-C/EBP-β signalling axis, activates the mTORC1 complex, which enhances drug tolerance in OSCC, etc. Cisplatin resistance is a major challenge in the treatment of OSCC. It is very important to further investigate the potential mechanisms of this resistance. Here, we use cell assays and clinical immunohistochemistry to examine the molecular pathways involved in both intrinsic and acquired cisplatin resistance. We demonstrate that the p62-mTORC1 signaling complex plays a crucial role and is driven by the EGFR signaling network, particularly through the PI3K-Akt axis and the transcription factor C/EBP-β. Elevated p-mTOR expression is associated with cancer recurrence and poor prognosis in oral cancer patients. Furthermore, mTOR inhibitors can enhance the cytotoxic effects of cisplatin by leveraging cancer stem cell properties [10].

4.3.6 MTMR6 Downregulation Leads to Cisplatin Resistance in OSCC.

Myotubularin-related protein 6 (MTMR6) plays a crucial role in cisplatin resistance in OSCC. Research has found that MTMR6 expression in cisplatin-resistant cells (UM-Cis) is significantly

lower than in the parental UMSCC1 cells. Functional assays confirmed that the regulation of MTMR6 activity directly affects resistance: overexpression of MTMR6 can enhance cisplatin sensitivity, while knockdown exacerbates resistance. Mechanistically, hsa-miR-544a inhibits the expression of MTMR6 by targeting its 3'UTR (validated by luciferase assays), and antagonizing this miRNA can restore MTMR6 levels and reverse resistance. Clinical analysis shows that high expression of MTMR6 in tumor tissues of OSCC patients is positively correlated with cisplatin sensitivity, while low MTMR6 expression in resistant patients' tissues and TCGA head and neck squamous cell carcinoma data indicates poor prognosis. In vivo experiments further confirmed that MTMR6 knockdown enhances the resistance of xenograft tumors to cisplatin treatment. This study is the first to reveal the molecular mechanism by which the MTMR6/hsa-miR-544a axis regulates cisplatin resistance in OSCC, suggesting that detecting the expression of MTMR6 and miR-544a could serve as biomarkers for predicting chemotherapy response. And provide new strategies for targeting this pathway (such as miRNA inhibitors) to enhance the efficacy of chemotherapy [11].

5. Conclusion

This article systematically summarizes the molecular mechanisms of cisplatin resistance in OSCC and the strategies for combined targeted therapy. The study found that resistance is driven by both intrinsic signaling abnormalities in tumor cells and TME remodeling. Targeting key molecules (such as inhibiting PD-L1 and blocking the PI3K/AKT pathway) or combining with immunotherapy (anti-PD1/PD-L1) can significantly reverse resistance, suggesting the potential of multi-target synergistic intervention. These findings provide important theoretical support for overcoming chemotherapy resistance in OSCC. The issues mentioned in the introduction, such as low selective toxicity and frequent resistance in chemotherapy, can potentially be addressed by targeting intrinsic signaling pathways and regulating the TME, thereby enhancing chemotherapy efficacy and reducing side effects. For example, molecules like CMTM6 and HPRT1 can serve as prognostic markers, while circ-ILF2 and miR-196a can be novel therapeutic targets. However, there are still limitations: most of the mechanisms are based on in vitro or animal models, and there is insufficient evidence for clinical translation; the interactions of TME components (e.g., Tregs, extracellular matrix) have not yet been fully resolved; there is a lack of multi-omics support for individualized therapeutic strategies, and spatial-temporal heterogeneity of the resistance mechanisms has not yet been fully explored.

Future research should focus on the following directions: conducting large-scale clinical trials to verify the synergistic effect of targeted drugs and chemotherapy; combining single-cell sequencing and spatial transcriptome technology to dynamically analyze the role of TME in drug resistance; developing prediction models based on artificial intelligence to screen sensitive patient populations; and exploring the emerging mechanisms of epigenetic and metabolic reprogramming in order to provide a more comprehensive strategy for combination therapy. Through multidisciplinary crossover and technological innovation, it is expected to break through the bottleneck of OSCC treatment and improve patient prognosis.

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