Gene Silencing Through RNA Interference in Oral Squamous Cell Carcinoma

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***Abstract*—Oral squamous cell carcinoma (OSCC) is the predominant histological subtype of oral cancer and remains a significant global health challenge. Conventional treatments, including surgery, radiotherapy, and chemotherapy, can cause substantial morbidity, while tumour recurrence, metastasis, and therapeutic resistance continue to affect clinical outcomes. RNA interference (RNAi) is an emerging molecular approach that enables sequence-specific regulation of gene expression and offers opportunities for targeted cancer therapy. This review examines the molecular mechanisms of RNA interference and evaluates its potential applications in OSCC, with particular emphasis on small interfering RNA (siRNA), microRNA (miRNA), oncogenic signaling pathways, targeted delivery systems, and translational limitations. RNAi-mediated gene regulation offers a potential strategy for suppressing oncogenic pathways associated with tumour proliferation, apoptosis resistance, invasion, metastasis, and treatment resistance. siRNA can selectively degrade complementary messenger RNA, whereas miRNA mimics and inhibitors can modify broader post-transcriptional regulatory networks. Lipid nanoparticles, polymeric nanocarriers, liposomes, and extracellular vesicles are being investigated to improve RNA stability and intracellular delivery. Nevertheless, inefficient tumour targeting, endosomal entrapment, off-target effects, immune activation, and limited OSCC-specific clinical validation remain major challenges. RNA interference represents a promising research direction for precision oncology in OSCC. Future progress will depend on validated molecular targets, effective delivery systems, comprehensive safety assessment, and rigorous clinical evaluation. At present, RNAi-based approaches should be regarded as investigational strategies rather than established treatments for OSCC.**

***Index Terms*—Oral squamous cell carcinoma; RNA interference; gene silencing; small interfering RNA; microRNA; oncogenes; tumour suppressor genes; nanocarriers; targeted therapy; precision medicine.**

I. INTRODUCTION

Oral squamous cell carcinoma is a malignant epithelial neoplasm arising from the mucosal lining of the oral cavity. It constitutes the majority of oral malignancies and is associated with substantial morbidity and mortality, particularly when diagnosis occurs at an advanced stage. Tobacco exposure, alcohol consumption, areca nut use, and other environmental and behavioural factors contribute to disease development, although their relative importance varies among populations. The biological behaviour of OSCC is influenced by genetic and epigenetic alterations affecting cell-cycle regulation, apoptosis, cellular differentiation, angiogenesis, invasion, and immune responses. Changes in signaling pathways such as epidermal growth factor receptor (EGFR), phosphoinositide 3kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR), and transforming growth factor-beta (TGF-β) can contribute to malignant progression and therapeutic resistance.[1] Current management strategies include surgical resection, radiotherapy, chemotherapy, and, in selected clinical settings, immunotherapy and molecularly targeted treatments. Although these approaches remain essential, treatment-related toxicity, functional impairment,

recurrence, and metastatic progression highlight the need for additional therapeutic strategies.

RNA interference is a conserved biological mechanism through which RNA molecules guide sequence dependent suppression of gene expression. Since its identification as a potent gene-silencing mechanism, RNAi has become an important research tool for investigating gene function and developing nucleic acid therapeutics.[2]

In OSCC research, RNAi can be used to investigate the contribution of specific genes to malignant behavior and to explore whether suppressing selected molecular targets improves sensitivity to existing therapies. However, sequence specificity does not guarantee biological specificity, and successful treatment requires efficient delivery to the appropriate tumour cells. This review discusses RNAi mechanisms, potential applications, delivery technologies, translational barriers, and future research priorities.

II. MOLECULAR MECHANISMS OF RNA INTERFERENCE

2.1 Overview of the RNAi pathway

RNA interference regulates gene expression through small RNA molecules that guide cellular protein complexes to complementary RNA sequences. In the canonical siRNA pathway, double-stranded RNA is processed or introduced as short RNA duplexes. One strand, known as the guide strand, is incorporated into the RNA-induced silencing complex (RISC). Argonaute 2 (AGO2), a catalytic component of RISC, can cleave a complementary target messenger RNA. The resulting RNA fragments are degraded, reducing production of the encoded protein.

The effectiveness of this mechanism depends on target-sequence accessibility, RNA stability, intracellular delivery, guide-strand selection, and target-transcript abundance.

2.2 Small interfering RNA

siRNA molecules are short double-stranded RNA sequences designed to recognize complementary regions of a selected messenger RNA. Following intracellular delivery, the guide strand associates with RISC and directs the complex towards the target transcript. In OSCC investigations, siRNA may be designed against genes involved in cellular proliferation, resistance to programmed cell death, tumour invasion and metastasis, angiogenesis, tumour–microenvironment interactions, or resistance to chemotherapy and radiotherapy.

Potential targets should be selected based on evidence of functional importance in the specific tumour model. Gene expression alone is insufficient to establish that silencing a particular target will produce a meaningful therapeutic effect.[3]

2.3 MicroRNA-mediated gene regulation

MicroRNAs are endogenous, short non-coding RNAs that regulate gene expression predominantly through interactions with partially complementary sequences in target messenger RNAs. They commonly repress translation and can promote messenger RNA destabilization. Unlike a typical siRNA designed against one target sequence, an individual miRNA can influence multiple transcripts and interconnected pathways.

In cancer, miRNAs may act as oncogenic miRNAs or tumour-suppressive miRNAs, depending on their targets and cellular context. Therapeutic approaches include miRNA mimics, intended to restore the activity of a miRNA whose expression or function is reduced, and miRNA inhibitors, designed to inhibit an overexpressed oncogenic miRNA. Because miRNAs frequently regulate multiple genes, their therapeutic use requires careful evaluation of intended and unintended molecular effects.[4]

2.4 Distinction between siRNA and miRNA approaches

Feature	siRNA	miRNA-based therapy
Principal objective	Silence a selected RNA transcript	Restore or inhibit a regulatory miRNA
Target recognition	Usually highly complementary	Often partial and context dependent
Typical effect	Targeted messenger RNA cleavage	Translational repression and/or RNA destabilization
Potential advantage	Relatively focused gene suppression	Modulation of interconnected pathways
Major limitation	Off-target silencing and delivery barriers	Broad target networks and context-dependent effects

III. MOLECULAR TARGETS AND THERAPEUTIC RELEVANCE IN OSCC

3.1 EGFR-associated signaling

EGFR signaling contributes to cellular proliferation, survival, migration, and tumour progression in several head and neck malignancies. Aberrant activation of this pathway can support malignant behaviour and influence treatment response. RNAi-mediated suppression of EGFR or selected downstream signaling components may help determine their functional contribution in OSCC models. Pathway redundancy and compensatory signaling can limit the effectiveness of inhibiting a single molecular target.

3.2 PI3K/AKT/mTOR signaling

The PI3K/AKT/mTOR pathway regulates cellular growth, metabolism, survival, and protein synthesis. Dysregulation of this pathway has been implicated in several cancers, including OSCC. RNAi directed against experimentally validated components may help clarify how pathway activity influences tumour cell viability, apoptosis, migration, and therapeutic resistance. Target selection should account for tumour genetics, pathway activation status, and possible effects on normal cells.[5]

3.3 Anti-apoptotic and cell-cycle regulatory genes

Cancer cells can acquire mechanisms that reduce susceptibility to apoptosis and maintain uncontrolled proliferation. Molecular regulators of apoptosis and the cell cycle are therefore important candidates for functional RNAi investigations. Silencing an anti-apoptotic gene may increase susceptibility to cell death, whereas suppressing a cell-cycle regulator may reduce tumour-cell proliferation. These effects must be confirmed experimentally because consequences can differ among OSCC models.

3.4 Invasion, metastasis, and epithelial mesenchymal transition

Tumour invasion and metastatic spread are associated with changes in cell adhesion, extracellular matrix remodeling, cellular motility, and epithelial–mesenchymal transition (EMT). RNAi studies can examine genes involved in EMT-associated signalling, matrix degradation, and cell migration. Reduced migration in a laboratory assay does not independently establish that a treatment prevents metastasis in patients.

3.5 Tumour-suppressive microRNAs

Tumour-suppressive miRNAs are being investigated for their ability to regulate oncogenic signalling, proliferation, invasion, and apoptosis resistance. Examples discussed in the broader cancer and OSCCrelated literature include miR-34a, miR-145, and miR-200-family members. Their effects depend on cellular context, target availability, and delivery strategy; activity should not be assumed to be equivalent across all OSCC subtypes.[6]

Table 1. Potential RNAi targets and proposed research outcomes

Molecular area	RNAi strategy	Outcome to investigate
EGFR signalling	siRNA against a validated target transcript	Proliferation and downstream pathway activity
PI3K/AKT/mTOR	siRNA against a selected pathway component	Survival, growth, and treatment response
Apoptosis regulation	siRNA against a validated antiapoptotic target	Apoptotic signalling and cell viability
EMT-associated pathways	siRNA against an invasion related target	Migration, invasion, and EMT markers
Tumour-suppressive miRNA loss	Delivery of a miRNA mimic	Restoration of regulatory activity and target suppression
Oncogenic miRNA overexpression	Delivery of a miRNA inhibitor	Reduced oncogenic miRNA activity and downstream effects

Note: The table describes research hypotheses and experimental endpoints. It does not imply that every listed strategy has demonstrated clinical efficacy in OSCC.

IV. DELIVERY SYSTEMS FOR RNAI-BASED OSCC THERAPY

4.1 The importance of delivery

RNA molecules are vulnerable to degradation by nucleases and may be unable to cross cellular membranes efficiently. After cellular uptake, therapeutic RNA can become trapped within endosomes and fail to reach the cytoplasmic machinery required for gene silencing. An effective delivery system must protect RNA, facilitate tumour-cell uptake, support intracellular release, and minimize exposure of healthy tissues.

The oral cavity presents additional considerations, including saliva, mucosal barriers, local inflammation, tissue heterogeneity, and the physical accessibility of individual lesions.

4.2 Lipid nanoparticles and liposomes

Lipid-based carriers can encapsulate or associate with RNA molecules and protect them from extracellular degradation. Appropriate formulations may improve cellular uptake and facilitate endosomal escape. Important limitations include formulation stability, distribution outside the intended tissue, inflammatory responses, and the need for reproducible manufacturing.[7]

4.3 Polymeric nanoparticles

Polymeric carriers can be engineered to regulate RNA loading, protect nucleic acids, and influence release kinetics. Their surface properties may be modified to improve cellular interactions or introduce targeting ligands. Their usefulness depends on biocompatibility, degradation characteristics, cargo release, and reproducibility.

4.4 Extracellular vesicles

Extracellular vesicles, including exosomes, are being investigated as potential carriers for therapeutic RNA. Potential advantages include biological compatibility and the possibility of engineering cargo loading or surface properties. Heterogeneity, purification, manufacturing scale-up, cargo quantification, and safety characterization remain important obstacles.

4.5 Local and systemic delivery

Local administration may be attractive for accessible oral lesions because it could increase drug exposure at the tumour site while limiting systemic exposure. However, local delivery may not adequately reach deep tumour margins, involved lymph nodes, or distant metastatic disease. Systemic delivery could address disseminated disease but requires sufficient tumour accumulation and acceptable toxicity.[8]

V. POTENTIAL THERAPEUTIC APPLICATIONS

5.1 Suppression of tumour-cell proliferation

RNAi can be used to investigate whether selected oncogenic transcripts are necessary for OSCC cell growth. Reduced proliferation following gene silencing may support further target validation, provided that findings are reproducible and not attributable to nonspecific toxicity.

5.2 Induction of apoptosis

Silencing genes that support tumour-cell survival may increase apoptosis under specific experimental conditions. Relevant outcomes include changes in caspase activity, apoptotic cell populations, and expression of apoptosis-associated proteins.[1]

5.3 Inhibition of migration and invasion

RNAi-based suppression of genes involved in motility, matrix remodelling, or EMT-associated signalling may reduce migration and invasion in cell-based assays. Further studies are required to determine whether these effects translate into reduced tumour dissemination in vivo.

5.4 Sensitization to chemotherapy and radiotherapy

A potential application of RNAi is the inhibition of molecular mechanisms that contribute to treatment resistance. Silencing a validated resistance-associated gene may increase OSCC-cell sensitivity to an established anticancer treatment. Combination studies must evaluate whether effects are additive, synergistic, or antagonistic and whether the combination increases damage to normal tissues.

5.5 Biomarker-guided precision treatment

OSCC is molecularly heterogeneous. Transcriptomic profiling, target-expression analysis, and functional assays may help identify tumour subgroups more likely to respond to a particular RNAi strategy. Clinically useful predictive biomarkers require independent validation.[9]

VI. EXPERIMENTAL FRAMEWORK FOR FUTURE RESEARCH

6.1 Target identification and validation

Candidate genes should be selected using literature evidence, tumour expression data, pathway analysis, and functional relevance. Where feasible, target expression should be compared between tumour and appropriate non-malignant tissue samples.

6.2 Selection of experimental models

Suitable models may include established OSCC cell lines, patient-derived tumour cells, three-dimensional cultures, organoids, and appropriately designed animal models. Multiple models can help determine whether an observed effect is robust or restricted to a particular genetic background.[10]

6.3 Experimental groups and controls

A typical preclinical design should include untreated or vehicle-treated controls, non-targeting siRNA or appropriate negative controls, candidate RNAi-treated groups, a positive control when scientifically appropriate, and non-malignant oral epithelial cells for selectivity assessment. For miRNA studies, the control strategy should account for the different mechanisms of mimics and inhibitors.

6.4 Outcome measurements

Relevant endpoints may include target messenger RNA abundance, target protein abundance, cell viability and proliferation, apoptosis and cell-cycle distribution, migration and invasion, pathway activity, cellular uptake and RNA localization, inflammatory responses, and toxicity. Quantitative reverse-transcription PCR, immunoblotting, flow cytometry, and microscopy may be used as appropriate.[4]

6.5 Statistical analysis and reproducibility

Experiments should include biological replicates, prespecified primary outcomes, appropriate statistical tests, and transparent reporting of effect sizes and uncertainty. Sample sizes should be justified according to the experimental design. Where possible, investigators should use independent siRNA sequences targeting the same gene and rescue experiments to distinguish sequence-specific effects from unintended silencing.

No experimental results are reported in this review. Any future original research article should include actual methods, measured data, statistical analyses, ethics approvals where applicable, and reproducible results.

VII. CHALLENGES AND LIMITATIONS

7.1 Off-target effects

RNAi molecules may influence unintended transcripts through partial sequence complementarity or other sequence-dependent interactions. Such effects can complicate interpretation and contribute to toxicity. Mitigation strategies include careful sequence design, multiple independent targeting sequences, transcriptome-wide assessment where feasible, and appropriate negative controls.

7.2 Inadequate intracellular delivery

Efficient delivery remains one of the most important barriers to RNAi therapy. Cellular uptake alone is insufficient if RNA remains trapped in endosomes or fails to engage the intracellular silencing machinery. Delivery systems should be evaluated for functional gene silencing, not merely for the presence of RNA within cells.

7.3 Immune activation and toxicity

Certain RNA sequences and delivery materials may stimulate innate immune responses. Toxicity can also arise from unintended gene suppression or accumulation in non-target tissues. Safety evaluation should include formulation-specific toxicity, immune activation, biodistribution, and potential effects on normal oral epithelium.

7.4 Tumour heterogeneity

OSCC lesions may contain cell populations with different genetic alterations, target-expression patterns, and treatment sensitivities. A molecular target that is essential in one tumour may be less important in another. Combination strategies or biomarker-based selection may help address heterogeneity, but they also increase development complexity.

7.5 Translation from laboratory models to clinical practice

Results obtained in cultured cells do not necessarily predict clinical outcomes. Animal models may also

fail to reproduce the full complexity of human tumour biology and immunity. Clinical translation requires evidence of appropriate dosing, reproducible delivery, target engagement, safety, and meaningful therapeutic benefit.

VIII. FUTURE PERSPECTIVES

Several research priorities could improve the translational potential of RNAi in OSCC. First, target selection should rely on functional evidence and molecular characterization rather than differential expression alone. Second, delivery technologies should be developed with explicit consideration of the oral tumour microenvironment, local administration, and systemic exposure.

Third, studies should incorporate models that better represent tumour heterogeneity, stromal interactions, and immune responses. Fourth, RNAi may be investigated in combination with established treatments when supported by a clear mechanistic rationale and appropriate safety assessment.

Finally, clinical development will require standardized manufacturing, stability testing, pharmacological characterization, regulatory oversight, and carefully designed clinical trials. Successful applications of RNA therapeutics in other diseases do not establish efficacy in OSCC; each candidate must be evaluated for its target, delivery system, tumour context, and clinical indication.

IX. CONCLUSION

RNA interference offers a valuable approach for investigating and potentially modulating the molecular pathways responsible for OSCC progression. siRNA enables sequence-directed suppression of selected transcripts, while miRNA-based approaches can alter broader gene-regulatory networks. Potential applications include inhibition of tumour-cell proliferation, induction of apoptosis, modulation of invasion-associated pathways, and investigation of treatment resistance.

Lipid-based carriers, polymeric nanoparticles, and extracellular vesicles may help overcome limitations of RNA stability and intracellular delivery. Nevertheless, off-target effects, immune activation, tumour heterogeneity, delivery inefficiency, and limited clinical validation remain significant barriers. Further progress requires rigorous target validation, disease-relevant experimental models, standardized safety assessment, and well-designed clinical studies.

RNAi should therefore be considered a promising investigational platform for precision oncology rather than an established treatment for OSCC. Translational success will depend on demonstrating that molecularly precise gene silencing can be delivered safely and produce clinically meaningful outcomes.

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