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MicroRNA-Mediated Regulation in Head and Neck Cancer: Current Insights and Future Perspectives

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Abstract

Head and neck cancers constitute a major global health burden, with Oral Squamous Cell Carcinoma (OSCC) representing the predominant histopathological subtype. Despite considerable advancements in therapeutic modalities, including surgery, radiotherapy and chemotherapy, the overall survival rate remains relatively poor, largely due to delayed diagnosis, tumour aggressiveness and frequent recurrence. In recent years, microRNAs (miRNAs) have emerged as important regulators of gene expression and have gained significant attention in cancer research.

MicroRNAs are small endogenous non-coding RNA molecules that regulate gene expression at the post-transcriptional level by interacting with target messenger RNAs, thereby modulating their translation and stability. Increasing evidence indicates that miRNAs play a pivotal role in the molecular pathogenesis of head and neck cancers by influencing critical cellular processes such as cell proliferation, apoptosis, differentiation, invasion and metastasis. Aberrant expression of several miRNAs, including miR-21, miR-31 and miR-34, has been reported in oral malignancies and is closely associated with tumour initiation and progression.

Furthermore, circulating miRNAs detected in biological fluids such as saliva, serum and plasma have demonstrated considerable potential as non-invasive biomarkers for the early detection, prognosis and therapeutic monitoring of Oral Cancer. Their remarkable stability and disease-specific expression profiles make them promising tools in precision oncology.

This review aims to provide a comprehensive overview of the biogenesis, detection methods and functional roles of microRNAs in head and neck cancer, with particular emphasis on their diagnostic, prognostic and therapeutic implications.

Keywords: MicroRNA, Oral Squamous Cell Carcinoma, Head and Neck Cancer, Biomarkers, miR-21, mir-31, mir-34.

Introduction

The control of when and where each gene should be transcribed from DNA to RNA and translated to protein is a fundamental aspect of life. In recent years, microRNAs (miRNAs) have emerged as important regulators of gene expression and have attracted significant attention in biomedical research [1]. MicroRNAs are small endogenous non-coding RNA molecules, approximately 19–25 nucleotides in length, that regulate gene expression at the post-transcriptional level by binding to complementary sequences in the 3′-untranslated regions (3′-UTR) of target messenger RNAs (mRNAs), leading to translational repression or degradation [2]. It is estimated that nearly 30% of human genes are regulated by miRNAmediated mechanisms [3]. The first microRNA, lin-4, was discovered in 1993 in Caenorhabditis elegans by Lee, Feinbaum and Ambros during studies on the gene lin-14, which controls larval developmental timing [4]. Instead of producing a protein-coding transcript, lin-4 generated a short non-coding RNA that bound to complementary sequences within the 3′-UTR of lin-14 mRNA, thereby inhibiting its translation. Parallel studies by Ruvkun and colleagues confirmed that lin-4 regulates developmental timing through repression of lin-14 expression [5]. Subsequently, the discovery of another conserved microRNA, let-7, by Reinhart and colleagues demonstrated that miRNA-mediated gene regulation is evolutionarily conserved across species [6]. Since then, numerous miRNAs have been identified that regulate key biological processes including cell proliferation, differentiation, apoptosis, stress response and immune regulation [7].

To date, approximately 2000 human miRNA precursor genes have been annotated in the miRNA database (miRBase) [8]. Dysregulation of miRNA expression has been implicated in several human diseases, particularly cancer, where certain miRNAs function as oncogenes while others act as tumour suppressors [9]. In head and neck cancers, especially Oral Squamous Cell Carcinoma, altered expression of miRNAs has been associated with tumour development, progression and patient prognosis[10].

Oral Squamous Cell Carcinoma (OSCC) is a malignant epithelial tumour characterized by squamous differentiation with keratin formation and/or the presence of intercellular bridges [11]. It is the most common malignancy of the oral cavity and forms part of the broader group of head and neck squamous cell carcinomas (HNSCC), which include cancers of the oral cavity, pharynx, hypopharynx, larynx, nasal cavity and salivary glands [12]. Collectively, these malignancies represent the seventh most common cancer worldwide[13]. According to GLOBOCAN estimates, approximately 890,000 new cases and 450,000 deaths occur annually due to head and neck cancers, with OSCC accounting for nearly 4.5% of global cancer diagnoses and mortality [13]. The disease predominantly affects men, with a male-tofemale ratio of about 2:1, and is most commonly diagnosed in individuals over 50 years of age [14]. The incidence is particularly high in India, where tobacco consumption, often combined with areca nut use, contributes to nearly 80% of cases [15].

Major etiological factors associated with OSCC include tobacco use (both smoked and smokeless), excessive alcohol consumption, high-risk human papillomavirus (HPV) infection, poor oral hygiene, and genetic and environmental factors [16]. The lateral border of the tongue is the most commonly affected site, followed by the

gingivobuccal sulcus (particularly in the Indian population), buccal mucosa, floor of the mouth, retromolar region, lip and palate [17].

Clinically, OSCC often presents as an ulcer with everted edges and indurated margins, frequently accompanied by fixation to underlying structures in advanced stages [17]. Most cases arise from pre-existing epithelial dysplasia of the oral mucosa, which represents the earliest histopathological stage with an increased risk of malignant transformation [18].

Histological features of epithelial dysplasia include nuclear pleomorphism, increased nuclearcytoplasmic ratio, nuclear hyperchromatism, atypical mitotic figures, irregular epithelial stratification, loss of basal cell polarity, drop-shaped rete ridges, premature keratinization, keratin pearl formation, and loss of epithelial cohesion [18]. Based on the extent of epithelial involvement, dysplasia is classified as mild, moderate or severe [18].

Despite advances in treatment, OSCC continues to show high morbidity and mortality, largely due to late diagnosis and early metastasis, with nearly 50% of patients presenting at advanced stages [19]. Early lesions are often asymptomatic and may mimic benign or inflammatory conditions, resulting in delayed diagnosis. Therefore, identifying reliable biomarkers capable of distinguishing oral potentially malignant disorders (OPMDs) from early OSCC is essential. In this context, microRNAs (miRNAs) have emerged as promising molecular biomarkers for early detection, risk assessment of malignant transformation and prognostic evaluation in Oral Cancer [20].

Discovery of MicroRNAs:

MicroRNAs (miRNAs) were first discovered in 1993 during studies on genetic regulation of developmental timing in the nematode Caenorhabditis elegans. Lee, Feinbaum, and Ambros identified a small non-coding RNA, lin- 4, which regulates the lin-14 gene by binding to complementary sequences within the 3′- untranslated region (3′-UTR) of its mRNA, thereby inhibiting protein translation [27]. Shortly thereafter, Wightman and colleagues further demonstrated that lin-4 regulates developmental timing through posttranscriptional repression of lin-14 [28].

Figure 1: Victor Ambros and Gary Ruvkun discovered microRNAs (miRNAs) in 1993 while studying how genes interact in the nematode C. elegans.

They won the 2024 Nobel Prize in Physiology or Medicine for their discovery of microRNAs.

For several years, this mechanism was believed to be unique to nematodes. However, in 2000, Reinhart and coworkers discovered another small RNA molecule, let-7, which was found to be evolutionarily conserved across multiple species[29]. This discovery established microRNAs as a widely conserved class of gene regulators and initiated extensive research into their roles in development, gene regulation, and human diseases, including cancer [30].

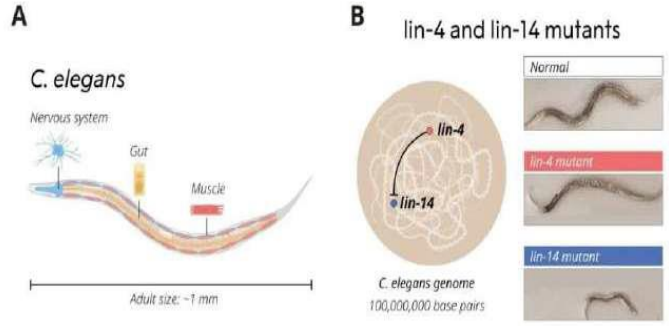


Figure 2 (A): C. elegans is a useful model organism for understanding how different cell types develop

Figure 2 (B) Ambros and Ruvkun studied the lin-4 and lin-14 mutants. Ambros had shown that lin-4 appeared to be a negative regulator of lin-14.

Biogenesis of MicroRNAs:

Following the discovery of microRNAs and recognition of their widespread role in gene regulation, considerable research has focused on understanding the mechanisms through which these small RNA molecules are generated and function within the cell. The production of mature microRNAs involves a complex and tightly regulated process that includes several enzymatic steps occurring in both the nucleus and cytoplasm. This process, known as microRNA biogenesis, ultimately leads to the formation of functional miRNAs capable of regulating gene expression through post-transcriptional silencing of target messenger RNAs. The biogenesis of microRNAs (miRNAs) is a highly regulated multi-step process involving both nuclear and cytoplasmic events that ultimately lead to post-transcriptional gene regulation.

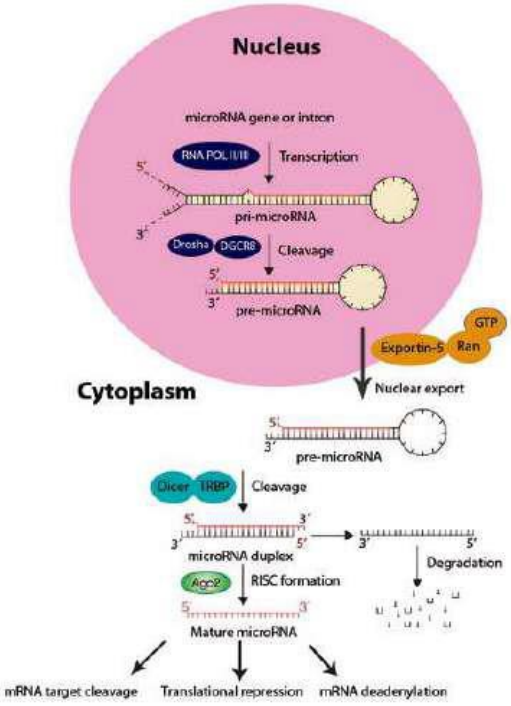


Figure 3: Biogenesis of micro RNAS

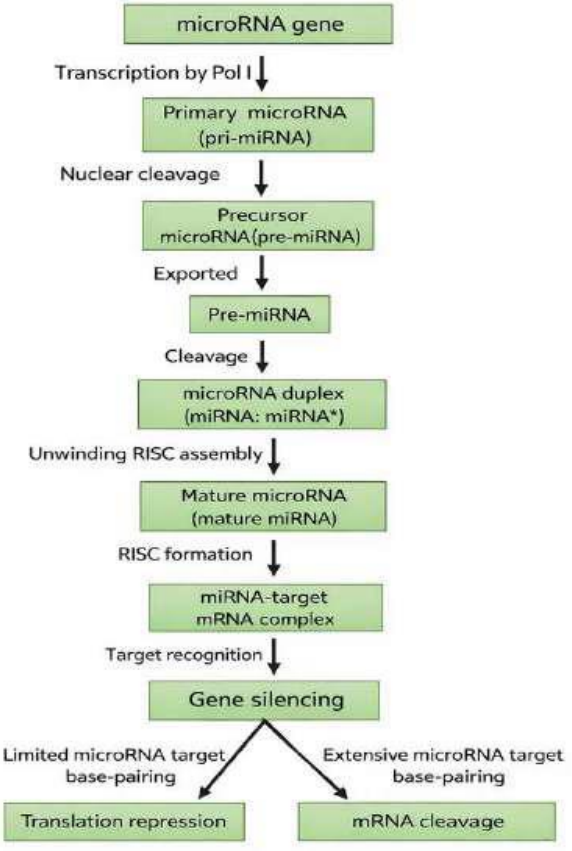


Figure 4: Canonical miRNA biogenesis and processing

01.Transcription:

MiRNA genes are primarily transcribed by RNA polymerase II, producing long primary transcripts known as primary miRNAs (pri-miRNAs). These transcripts may consist of several hundred nucleotides and contain characteristic stem-loop hairpin structures [21].

02. Nuclear Processing:

Within the nucleus, pri-miRNAs undergo cleavage by the Drosha–DGCR8 microprocessor complex, generating a precursor miRNA (pre-miRNA) of approximately 60–70 nucleotides in length [22].

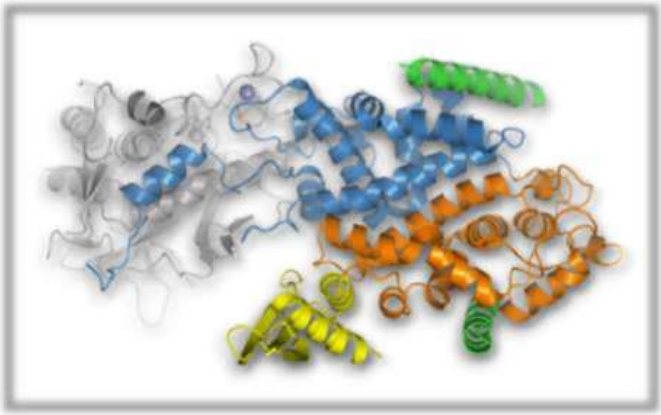


Figure 5: A crystal structure of the human Drosha protein in complex with the C-terminal helices of two DGCR8 molecules (green).

03. Nuclear Export: The resulting pre-miRNA is transported from the nucleus to the cytoplasm through Exportin-5, a transport receptor that functions in a Ran-GTP– dependent manner [23].

04. Cytoplasmic Processing: Once in the cytoplasm, the RNase III enzyme Dicer further processes the pre-miRNA into a double-stranded miRNA duplex, typically about 22 nucleotides long [24].

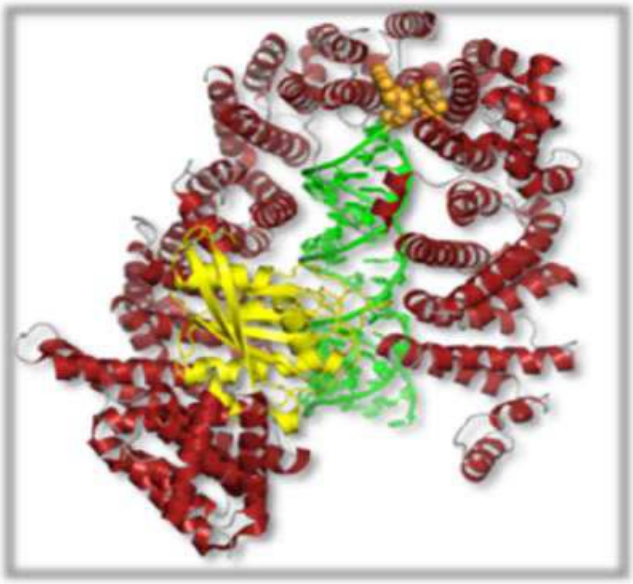


Figure 6: The human exportin-5 protein

05. Formation of RNA-Induced Silencing Complex (RISC): One strand of the miRNA duplex, known as the guide strand, is incorporated into the RNAinduced silencing complex (RISC), which contains Argonaute proteins, particularly AGO2. The other strand, known as the passenger strand, is usually degraded [25].

06. Gene Silencing: The miRNA-RISC complex binds to complementary sequences within the 3′ untranslated region (3′-UTR) of target messenger RNAs (mRNAs), resulting in either translational repression or degradation of the target mRNA. Through this mechanism, miRNAs are estimated to regulate approximately 30–60% of protein-coding genes in humans [26].

MicroRNA Isolation:

MicroRNAs (miRNAs) can be isolated from cells, tissues, and body fluids such as serum, plasma, tears, and urine. Early studies commonly used phenol–chloroform extraction followed by RNA precipitation, typically with TRIzol reagent. However, this method may introduce contaminants and lead to loss of small RNAs, particularly miRNAs with low guanine–cytosine content [31].

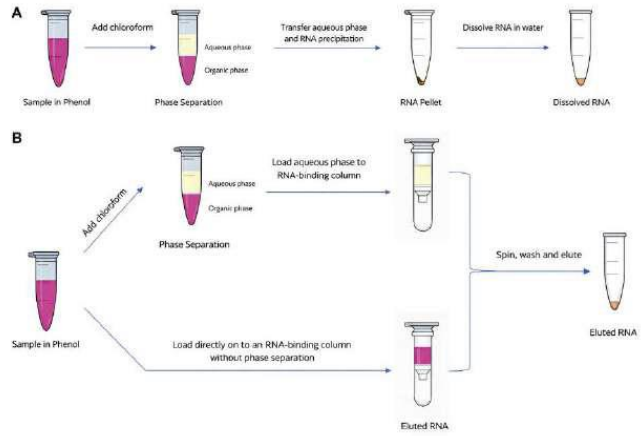


Figure 7: Isolation method for MicroRNAs

To improve yield and purity, column-based RNA adsorption methods are widely used. In this approach, RNA from the aqueous phase is loaded onto an adsorption column, followed by washing and elution. Commercial kits such as mirVana and miRNeasy are commonly used, while newer kits like Direct-zol allow direct loading of phenol-containing samples without phase separation [31].

Isolation of miRNAs from body fluids is more challenging due to their low abundance, often requiring larger sample volumes for effective recovery [31].

Detection of MicroRNAs: Accurate detection and quantification of microRNAs (miRNAs) are essential for understanding their biological roles and potential clinical applications. Several molecular techniques have been developed for miRNA detection, each with varying sensitivity and specificity.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) is the most commonly used method due to its high sensitivity, specificity, and ability to quantify miRNA expression levels in tissues and body fluids [32].

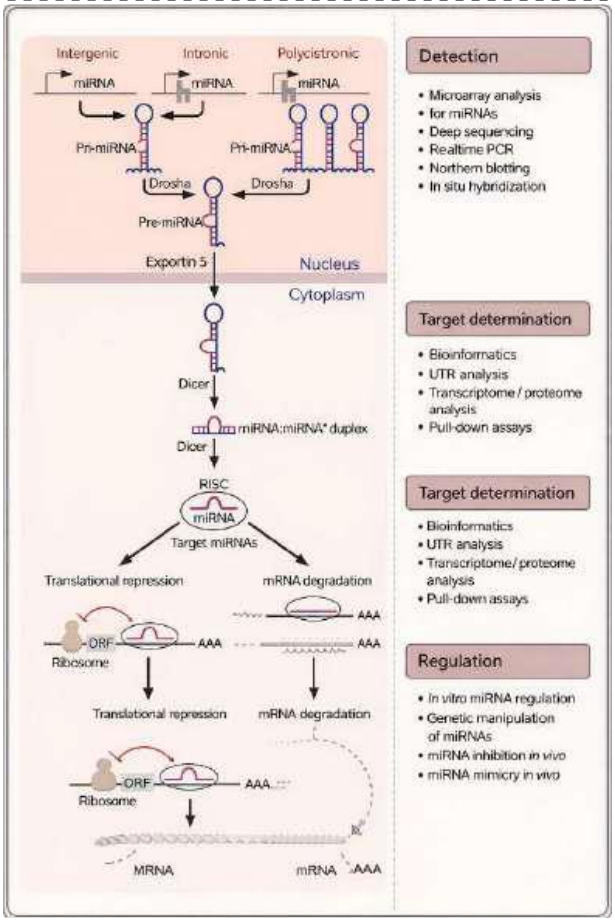


Figure 8: miRNA biogenesis and research tools

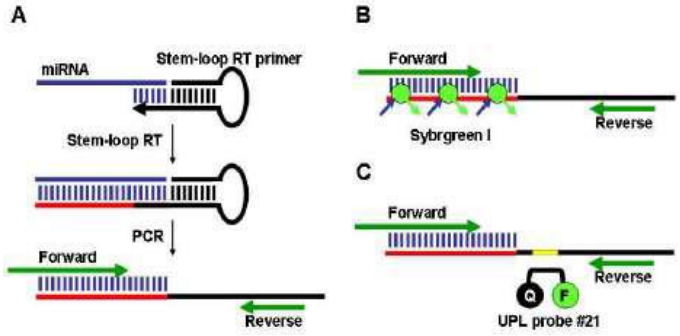


Figure 9: Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Microarray analysis enables simultaneous profiling of large numbers of miRNAs and is useful for identifying differential expression patterns in normal and diseased tissues [33].

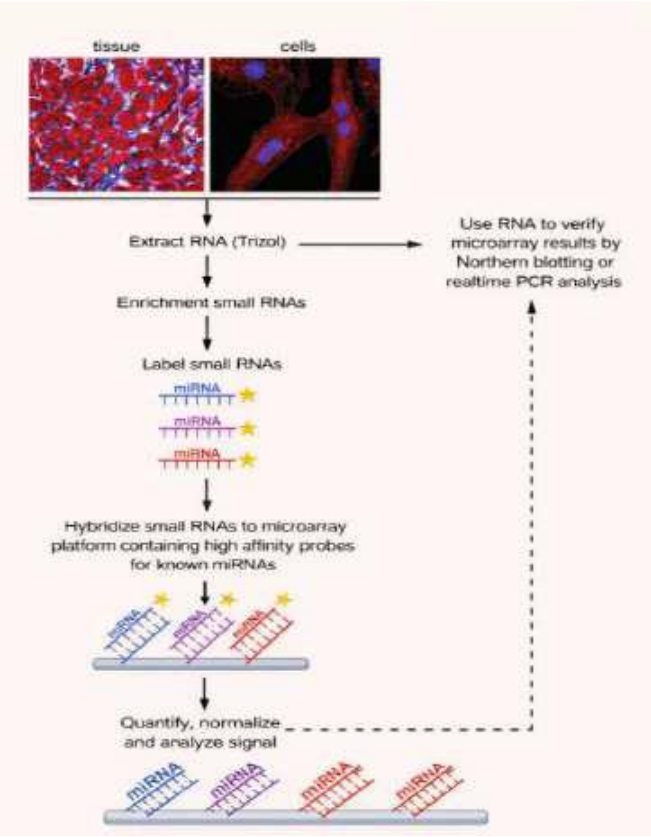
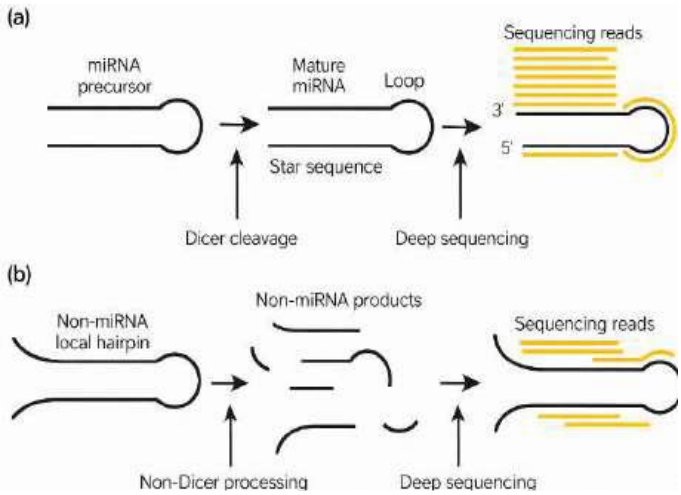


Figure 10: Microarray analysis

More recently, next-generation sequencing (NGS) has been employed for comprehensive analysis of known and novel miRNAs, providing highly accurate expression profiling [34].



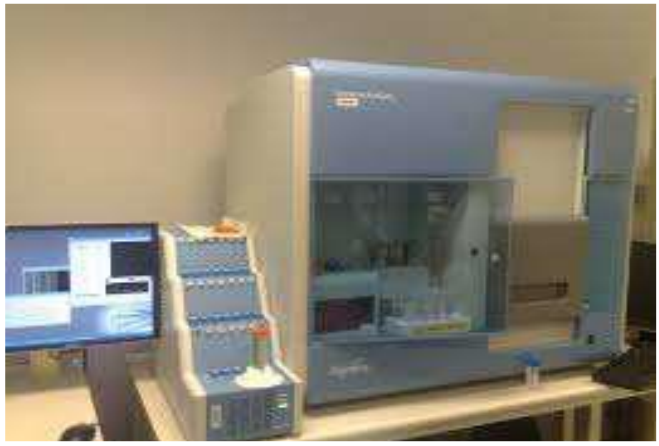


Figure 11: (a) Analysing the compatibility of sequenced RNAs with miRNA biogenesis,

(b) Illumina Genome Analyzer: a pioneering next-generation sequencing (NGS) platform that revolutionized genomics with high-throughput, short-read sequencing (36–150 bp) using reversible dyeterminator technology.

Additionally, Northern blotting and in situ hybridization are used to detect and localize miRNA expression within tissues, although these methods are less sensitive compared with modern molecular techniques [35].

Dynamics of Micro RNA action:

MicroRNA-Mediated Gene Silencing via miRISC

The minimal microRNA-induced silencing complex (miRISC) consists of the guide miRNA strand bound to an Argonaute (AGO) protein [39]. Target recognition occurs through binding of the miRNA to complementary sequences on messenger RNA known as microRNA response elements (MREs). The degree of complementarity between the miRNA and the MRE determines the mechanism of gene silencing. Perfect or near-perfect complementarity induces AGO2-mediated endonucleolytic cleavage of the target mRNA, whereas partial complementarity usually results in translational repression and mRNA degradation [36,38].

In animal cells, most miRNA–MRE interactions are partially complementary, typically containing

mismatches that prevent AGO2-mediated cleavage. In these cases, AGO proteins function primarily as mediators of RNA interference. Effective target recognition generally depends on the 5′ seed region of the miRNA (nucleotides 2–8), while additional pairing at the 3′ region enhances the stability and specificity of the interaction [36,37,38].

Following target recognition, miRISC recruits members of the GW182 protein family, which act as scaffolding proteins for the assembly of additional effector complexes. These include the PAN2–PAN3 and CCR4–NOT deadenylase complexes, which initiate removal of the poly(A) tail of the target mRNA. Interaction between GW182 and poly(A)-binding protein (PABPC) facilitates efficient deadenylation. Subsequent decapping of protein 2 (DCP2) allows degradation of the mRNA by the 5′–3′ exonuclease XRN1, ultimately resulting in gene silencing [36,37,38].

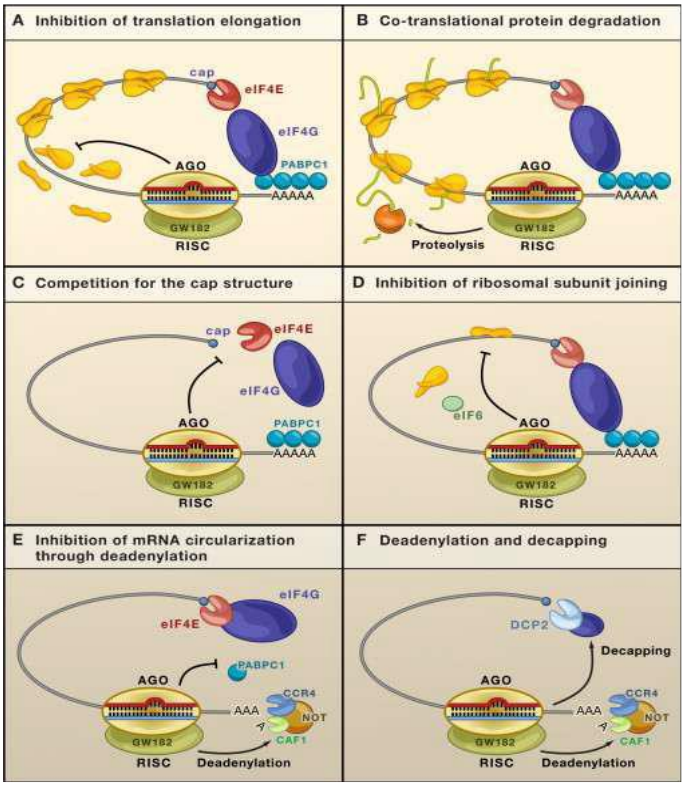


Figure 12: Mechanisms of miRNA-Mediated Gene Silencing

MicroRNA expression in normal tissues refers to the presence and levels of microRNAs (miRNAs) found in different healthy human tissues. MicroRNAs are small, non-coding RNA molecules, usually about 20–24 nucleotides in length, that regulate gene expression at the posttranscriptional level. They function by binding to target messenger RNAs (mRNAs), leading to either the degradation of the mRNA or the inhibition of its translation into proteins. In normal human tissues, miRNAs exhibit distinct expression patterns: some are expressed broadly across many tissues, while others are specific to particular tissues. For instance, miR-1 is predominantly expressed in muscle tissue, miR-122 is highly abundant in the liver, and miR- 124 is mainly found in the brain. These specific expression patterns play a crucial role in regulating various biological processes, including cell differentiation, development, metabolism, and maintenance of normal cellular functions. Understanding microRNA expression in normal tissues provides a fundamental reference for studying gene regulation and helps researchers compare normal physiological conditions with disease states, thereby aiding in the identification of potential biomarkers and therapeutic targets.

Role of MicroRNAs in Head and Neck Cancer:

MicroRNAs (miRNAs) play a crucial role in the regulation of multiple cellular pathways involved in tumourigenesis, including cell cycle regulation, apoptosis, angiogenesis, and metastasis. Aberrant expression of miRNAs has been reported in several malignancies, including head and neck squamous cell carcinoma (HNSCC).

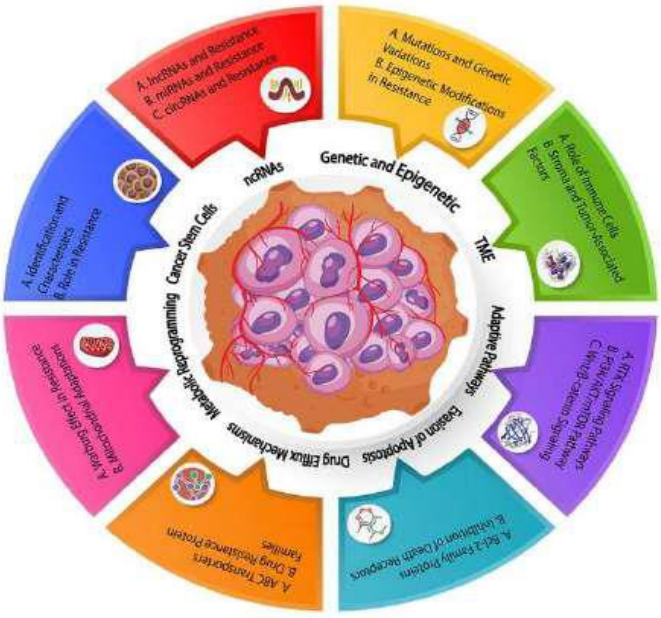


Figure 13: Hallmarks of Cancer

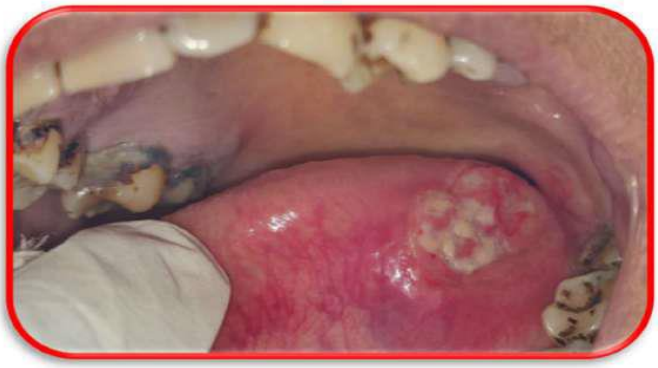


Figure 14: OSCC on the ventro lateral border of left side of tongue

Depending on their targets, some miRNAs function as oncogenes (oncomiRs) that promote tumour development, whereas others act as tumour suppressors that inhibit cancer progression.

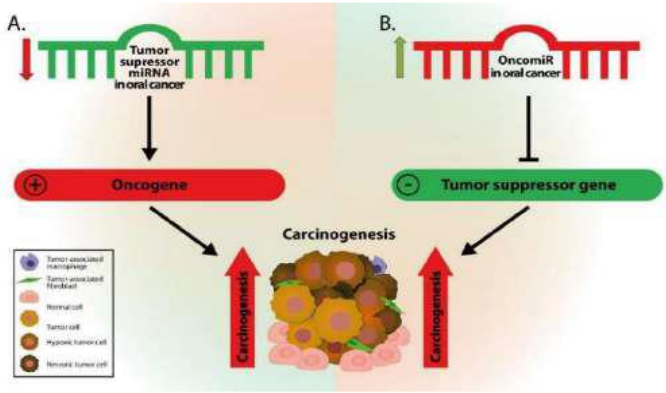


Figure 15: Two types of microRNAs in Oral Cancer

a) miR-21 in Oral Cancer:

Among the oncogenic miRNAs, miR-21 is one of the most extensively studied and is frequently overexpressed in various cancers such as breast, lung, colorectal, and head and neck cancers. In oral squamous cell carcinoma (OSCC), miR-21 expression is significantly elevated compared with normal oral mucosa. Overexpression of miR-21 promotes tumour growth and progression by inhibiting tumour suppressor genes including PTEN and PDCD4 [15].

Increased miR-21 expression has also been observed in premalignant lesions such as Oral Leukoplakia with dysplasia, suggesting its potential role as an early biomarker for malignant transformation. Furthermore, high miR-21 levels have been associated with advanced TUMOUR stage and poor prognosis in OSCC patients.

miR-21 is a small regulatory microRNA that helps control gene expression after transcription. It is first produced as pri-miR-21, which is processed in the nucleus by the enzyme Drosha to form pre-miR-21. This precursor is then transported to the cytoplasm by Exportin-5, where another enzyme, Dicer, cuts it to generate the mature miR-21 molecule. The mature miR-21 becomes part of the RNA-induced silencing complex (RISC), which guides it to bind to the 3′- UTR of specific target mRNAs, including tumour-suppressor genes such as PTEN and PDCD4. By binding to these mRNAs, miR-21 either blocks their translation or promotes their degradation. As a result, the levels of tumour-suppressor proteins decrease, which can enhance cell proliferation, invasion, and resistance to apoptosis, contributing to cancer development.

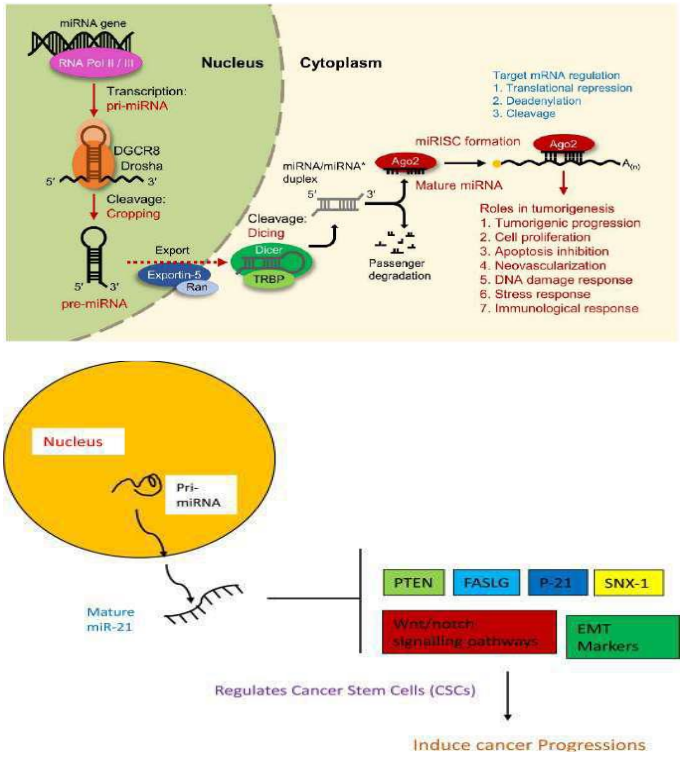


Figure 16: (a) &(b) Role of Micro RNA 21 in cancer progression

b) miR-31 and Oral Carcinogenesis: miR-31 plays an important role in cancer progression, particularly under hypoxic (low oxygen) conditions within the tumour microenvironment. Hypoxia activates Hypoxia-Inducible Factor- 1 alpha (HIF-1α), a transcription factor that regulates genes involved in TUMOUR survival, angiogenesis, and metabolism.

Under hypoxic conditions, HIF-1α increases the expression of miR-31 by binding to hypoxiaresponsive elements in the promoter region of the miR-31 gene. The elevated miR-31 then regulates several target genes involved in cell migration, invasion, and metastasis. One of the important targets of miR-31 is FIH (Factor Inhibiting HIF-1), a negative regulator of HIF-1α. By suppressing FIH, miR-31 enhances the activity of HIF-1α, creating a positive feedback loop that further strengthens hypoxia signalling.

As a result, increased miR-31 activity leads to:

- enhanced angiogenesis
- increased tumour cell proliferation
- greater migration and invasion
- promotion of metastasis

Therefore, the interaction between miR-31 and HIF-1α contributes significantly to tumour progression, especially in cancers such as oral squamous cell carcinoma, breast cancer, and lung cancer, where hypoxia is a common feature of the tumour environment [16].

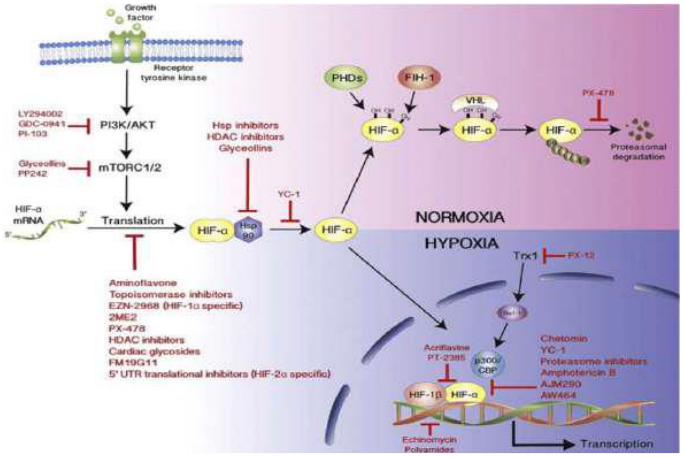


Figure 17: Mechanism of action of the HIF inhibitors, blocking different steps of HIF signalling pathway

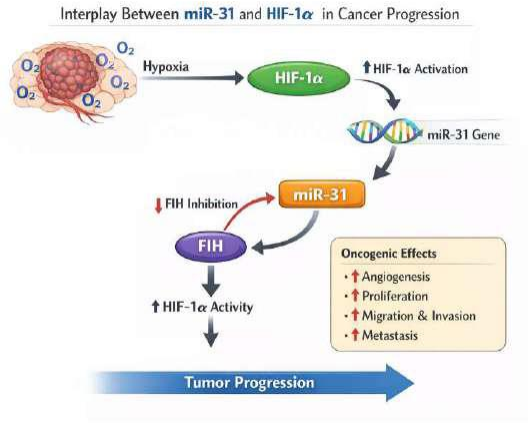


Figure 18: Mechanism of mir 31 in Cancer progression

c) Tumour Suppressor MicroRNAs: miR-34

The miR-34 family functions as a tumour suppressor and is directly regulated by the p53 tumour suppressor pathway. miR-34 plays a critical role in controlling cell cycle arrest, apoptosis, and inhibition of tumour growth. Reduced or lost expression of miR-34 has been reported

in several cancers, including head and neck carcinoma. Restoration of miR-34 expression has been shown to suppress tumour proliferation and induce apoptosis, highlighting its potential as a therapeutic target in cancer treatment [17].

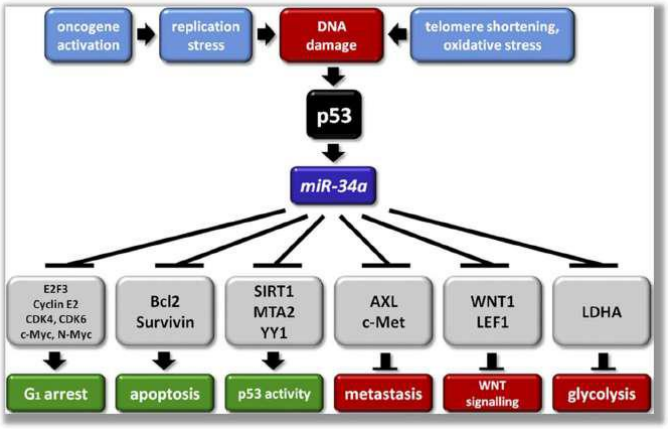


Figure 19: Key Concept: p53 → activates miR-34 → suppresses oncogenic genes → induces apoptosis and cell cycle arrest.

Therapeutic Aspects of MicroRNAs:

MicroRNAs (miRNAs) have emerged as promising therapeutic targets in cancer due to their ability to regulate multiple genes involved in tumour progression. One potential therapeutic strategy involves modulating dysregulated miRNA levels, either by restoring downregulated tumour-suppressor miRNAs using miRNA mimics or by inhibiting overexpressed oncogenic miRNAs using antisense oligonucleotides (antagomiRs).

The first clinical application of miRNA-based therapy was demonstrated in patients with chronic hepatitis C virus (HCV) infection. Miravirsen, an antisense oligonucleotide targeting miR-122, was developed to inhibit viral replication since miR-122 plays an essential role in HCV propagation. Clinical trials have shown promising antiviral activity and safety, highlighting the therapeutic potential of miRNA-targeted approaches[1,3,13,19].

In oncology, MRX34, a synthetic mimic of the tumour-suppressor miR-34, represents one of the first miRNA-based therapeutic agents evaluated in cancer patients. miR-34 is frequently downregulated in various tumours and functions by regulating oncogenic pathways involving MYC, MET, BCL2, and β-catenin. A phase I clinical trial investigated the safety, pharmacokinetics, and maximum tolerated dose of MRX34 in patients with primary liver cancer and liver metastases [1,3,13,19].

Several experimental studies have also demonstrated the therapeutic relevance of specific miRNAs in Oral Squamous Cell Carcinoma (OSCC). Upregulation of miR-375 has been shown to inhibit cell proliferation, induce G0/G1 cell cycle arrest, promote apoptosis, and enhance radiosensitivity in OSCC cells. Similarly, miR-148a suppresses migration and invasion of oral cancer cells by directly targeting WNT10B. Other miRNAs such as miR-1254, miR-377, and miR-23a-3p have also been reported to inhibit tumour cell proliferation and promote apoptosis, indicating their potential as therapeutic targets for OSCC.

Overall, miRNAs represent promising molecular targets for the development of novel anticancer therapies, offering potential strategies for targeted treatment and improved clinical outcomes in oral cancer [1,3,13,19].

Conclusion

MicroRNAs (miRNAs) have emerged as crucial regulators of gene expression and play a fundamental role in maintaining cellular homeostasis by modulating processes such as cell proliferation, differentiation, apoptosis, and stress responses. Since their discovery, miRNAs have significantly expanded our understanding of the molecular mechanisms underlying numerous diseases, particularly cancer.

Accumulating evidence indicates that aberrant miRNA expression contributes to the initiation, progression, and

metastasis of head and neck cancers, including oral squamous cell carcinoma (OSCC). Several miRNAs function either as oncogenes or tumour suppressors, influencing multiple signalling pathways involved in tumour development. Consequently, miRNAs have attracted considerable attention as potential biomarkers for early diagnosis, prognosis, and therapeutic targets in cancer.

Recent advances in molecular research have highlighted the therapeutic potential of miRNA based strategies, including the use of miRNA mimics to restore tumour suppressor miRNAs and inhibitors to suppress oncogenic miRNAs. Although several challenges remain, such as efficient delivery systems and minimizing off-target effects, ongoing research and clinical studies continue to improve the feasibility of miRNA-based therapies.

In particular, circulating miRNA signatures associated with head and neck carcinoma may serve as valuable diagnostic and prognostic biomarkers, providing insights into disease stage, patient survival, and therapeutic response. With continued research, miRNA-based diagnostics and therapeutics may play a transformative role in the development of precision medicine approaches for Oral Cancer management.

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