Imprints of miRNA- An Update

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Abstract

Introduction: Micro Ribonucleic acid (miRNA) is the novel fraction of non-coding RNA, which is considered as the biological measure of genetic state of a cell. It plays a mantle regulatory role post transcriptionally, involving both biological and pathologic cellular processes. miRNAs possess unique properties like, abundant tissue expression which allows for reproducible isolation and quantification.

Methods: Literature search was carried out by using different combinations of keywords on Pub Med and Cochrane database to retrieve the data for the literature review.

Discussion: Distinct expression profiles of miRNA in OSCC (Oral Squamous Cell Carcinoma) offers the use of specific miRNAs signature for early-stage diagnosis, prediction and prognosis. It plays a critical role in the initiation and progression of cancer functioning either as an oncogene or as tumor suppressor. At present, it has a huge impact on understanding the aberrant gene modifications in OSCC and its future effects on prognosis and therapeutic relevance needs to be looked upon.

Conclusion: Distinct phases of miRNA from its origin, properties, functionality, detection, cancer association to future trends are updated in this review.

Keywords: *miRNA, MicroRNA, Profiling, Biomarker, Oral cancer*

Introduction

Epigenetic phenomenon is a novel thread, linking enormously to the process of carcinogenesis in oral cancer. One of the most recent entrants in the category of epigenetic profiling is a single stranded endogenous, non-coding RNA and is referred to as micro RNA (miRNA). These are a group of gene-specific regulators which are involved in many essential biological activities such as cellular differentiation, proliferation, and apoptosis and thus, their deregulation can affect normal cell growth and even participate in carcinogenesis. (1)

History of miRNA:

- The initial discovery of first mi RNA dates back to 1993, when lin-4, a small non coding RNA was associated to the developmental timing of the nematode *Caenorhabditis elegans by* Ambros and coworkers.
- The second miRNA, let-7 was identified from C.elegans in 2000. (2)

Glossary of miRNA(3):

- The prefix **"miR"** is followed by a dash and a number Eg: miR-21
- Capital **R** refers to mature form Eg: miR

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- Uncapitalised **"mir"** refers immature forms.

- Primary **(pri-mir)** and Precursor **(pre-mir)** Eg: pri-mir-16, pre-mir-16.

- **a or b:** Represents isoforms which are closely related in a sequence. Eg: miR-26a

- **Suffix -3p or -5p:** Mature miRNA originating from the 3' or 5' end of the pre-miRNA respectively. Eg: miR-142-3p, miR-142-5p.

- **-1 or -2:** Identical mature miRNA sequences that originate from different genomic loci.

- **MI RNA** refers to genes that encode them

- **miR* :** An asterix indicates the passenger strand found in lower concentration. Eg: miR-9*

- **hsa and dme:** Homo sapiens and fruitfly

- **v** : miRNA encoded by viral genome

- **d**: drosophila

- **cel :** Caenorhabditis elegans

- **ath:** Arabidopsis thaliana (4)

Characteristics of miRNA:

- Novel class of endogenous, non-protein coding small RNA molecule. (5)

- Evolutionarily conserved, naturally abundant, relatively stable molecules. (6)

- It represents 0.01% of total RNA by weight (3)

- Total no of mature miRNAs in humans is estimated as 2580. (7)

- It requires partial sequence homology to 3' UTR (Untranslated Region) of target gene. Because of this unique feature of miRNA targeting, single miRNA can have multiple targets and thus could directly regulate a large no of protein coding genes. (8)

- It is a key regulator of gene expression and is estimated to regulate 30 % of human genes. (9)

- Multiple miRNAs can target one mRNA, while one mRNA can influence mRNA transcripts of numerous genes. (10)

- It has low molecular weight and is much more stable compared to mRNA (3)

- The melting temperature (Tm) of miRNA is an important biochemical property in the profiling. (5) Most miRNAs are predicted to have a Tm temperature of 55 °C. (11)

- Few miRNAs are tissue specific and differential expression of specific miRNA has been reported. Some miRNAs exhibit strong enrichment in restricted tissues. (11)

- It can be intracellular or extracellular. (13)

- miRNA exist in three forms: Short mature, Hairpin pre-miRNA and Long pri-miRNA. (11)

Synthesis of miRNA: Biogenesis of miRNA is due to two-fold influence from cytoplasm and nucleus of a cell. In the nucleus, miRNA's are transcribed from the genome as individual transcripts or as clusters. These primary miRNA (Pri-miRNA) transcripts are processed by ribonuclease III-Drosha and its cofactor Pasha, into precursor form (pre-miRNA). (14) At this level, hairpin structure of primary form is processed into 60-70 nts long Pre-miRNA. **(15)** With the help of exportin 5, precursor miRNAs are exported to cytoplasm and cleaved by dicer which generates a dsRNA (double stranded) of approximately 22 nts, named miR/miR*.

Then, this mature single stranded miRNA is incorporated into the miRNA induced silencing complex (miRISC), where miRNA acts as a guide to target messenger RNA for selective degradation or translational suppression. (14)

Structure and Functions of mi RNA: miRNA is processed from stem-loop precursors (11) (Table 1).

Table 1: Structure of miRNA

Properties	Pri –miRNA	Pre-miRNA	Mature miRNA
Length	Several 100 nts	60-70 nts	19-24 nts
Structure	Hairpin shape	Stem loop structure	Double strand linear (Guide & Passenger strand)
Location	Processed in Nucleus	Processed in Cytoplasm	Processed in Cytoplasm

These are the epigenetic modulators which control the gene expression by two distinct mechanisms namely, degradation of target mRNA transcripts and inhibition of mRNA translation.

miRNAs regulate gene in both biological and pathological processes. **(2, 5, 6, 8, 9)**

- Regulate genes related to cell differentiation, proliferation and apoptosis
- Developmental timing and metabolism
- Regeneration & Neuronal differentiation
- Regulation of immune system.
- Signaling pathway
- Oncogenesis and metastasis
- Forensic diagnostics

miRNAs are abundantly expressed in tissues (Fresh tissue, Formalin fixed, paraffin embedded tissue), cell lines, saliva and urine. (3) Circulating miRNAs are identified in blood plasma, platelets, leukocytes and erythrocytes. (6)

Methods of detection of miRNA: miRNA profiling is defined as "the measurement of the relative abundance of cohort of miRNAs, ranging from a group of several miRNAs of specific biological interest, to comprehensive profiling of all miRNAs in a given species". (3)

Evolution of miRNA is an ongoing process and thus various technologies are available for miRNA expression profiling. Many techniques have evolved significantly over the years.

Working Classification on methods to detect miRNA (11):

I Based on RNA sample amplification :

1. Amplification
   - QRT-PCR (Quantitative Reverse Transcriptase Polymerization Chain Reaction)
2. Hybridization
   - In-situ hybridization
3. Combination of amplification & Hybridization
   - Bead based flow cytometry method
   - RAKE (RNA primed Array based Klenow Enzyme assay)
   - MIRAGE (miRNA analysis of gene expression)

II Based on throughput (6)

1. High
2. Low

III : Based on location(13) **:**

1. Intracellular
2. Extracellular

Oral Cancer perspective of miRNA: miRNAs play an important regulatory role during carcinogenesis. Recent evidence suggests that deregulation of miRNA genes play an important role in HNSCC (Head and neck squamous cell carcinoma)

A. miRNA in transition from Dysplasia to OSCC: Few miRNA studies investigated the deregulation of miRNAs in the transition process from dysplasia to malignancy.

· Up-regulation is noted in **miR-31, miR-31*** and Down-regulation of **miR-375** was noted in a study.

· It was also found that **miR-21, miR-181b and miR-345** were consistently increased in oral dysplasia and had highly significant associations with progressive leukoplakia lesions and played a role in malignant transformation. (10)

B. miRNA in OSCC: Approximately 30-50 miRNAs are linked to OSCC and many miRNAs with potential biological and clinical relevance have been identified and are being explored as diagnostic, prognostic and predictive markers. (15)

· **miR-21:** It is the most extensively studied miRNA exhibiting proto-oncogene properties. It is over expressed in HNSCC through the regulation of clusterin and is strongly correlated with the progression of premalignant leukoplakia to invasive oral carcinoma. (15) miR-21 functions as an oncogene and is considered an independent factor of disease-free survival in OSCC. Up-regulation leads to inhibition of apoptosis, while its down-regulation leads to chemo resistance. It serves as both diagnostic and prognostic marker. Relationship between miR-21 and PNI (Perineural Invasion) in OSCC is also identified. (16)

· **miR-221:** It acts as oncomir and is known to suppress p27 and p57 transcripts of the retinoblastoma tumor suppressor family. It is over-expressed in HNSCC, confirming its role in oral carcinogenesis. (15)

· **miR-155:** It is up-regulated in OSCC in comparison with normal tissue and is down- regulated in the presence of HPV-16 in a series of HPV-positive cell lines of the head and neck. It is characteristically up-regulated in early stage of OSCC. (15)

· The ratio of **miR-221: miR-375** may be significant in discerning malignant HNSCC from normal tissue. **(10)**

· **miR-26a and miR-26b** are significantly down regulated in OSCC and its restoration in cancer cell lines revealed that, these miRNAs inhibited cancer cell migration and invasion. (17)

· **miR-16** is up-regulated in OSCC cell lines and tumor tissues. (18)

· Reduced expression of **Let-7 family** (let-7a, let-7b and let-7d) miRNA is a characteristic feature of HNSCC as it regulates the expression of Ras and other genes involved in the cell cycle, thereby repressing cell division and is thought to negatively modulate EMT

(Epithelial Mesenchymal Transition) in OSCC cell lines. (18)

· Over expression of **miR-142-3p and miR-29b** is observed in OSCC. (18)

· **miR-144** is frequently up-regulated in nasopharyngeal carcinoma and 8-fold up-regulation in OSCC. (18)

· **mir-148b-3p, miR-629-3p, miR-27a-3p and miR-142-3p** are screened as novel diagnostic biomarkers for OSCC based on their unique regulatory abilities. (17)

C. miRNAs in Metastases :

· **miR-205** expression is down regulated in lymph node metastases. (10)

· **miR-134** is up-regulated in HNSCC tissues relative to control mucosal tissue and is associated with nodal metastasis and mortality of patients. (16)

D. miRNAs in body fluids: miRNA is resistant to RNase activity and may be universally present in cell-free body fluids. It is reported that miRNAs are extremely stable in serum and plasma.

· Amplified plasma levels of miR-184, miR-31 and miR-24 have been detected in OSCC in comparison with control tissue.

· MiR-125a and miR-200a are the salivary markers which are shown to be substantially reduced in OSCC.

· Plasma and salivary miR-31 and plasma miR-184 are shown to be reduced after tumor resection implying that these miRNAs are released from cancerous tissue into circulation. (10)

E. miRNA in Surgical margins: Decreased expression of miR-125a, miR-184, miR-16 and increased expression of miR-96 in both progressive oral mucosal samples and dysplastic surgical margin samples are investigated in a study by Santhi et al. (10)

F. miRNA in HPV (Human Papilloma Virus) associated HNSCC:

· miR-15a, miR-16, miR-195, miR-497, miR-143 mir-145 and miR-106-363 cluster are a set of core miRNAs implicated in the HPV+ HNSCC. miR-9, miR -223, miR -31, miR -18a and miR -155 are also investigated in HPV positive oropharyngeal SCC (Squamous Cell Carcinoma).

· Increased expression of miR-375 is observed in pharyngeal and laryngeal tumors unassociated with HPV. (10)

G. miRNAs and associated Signal pathways: Recent studies have identified a novel SCC molecular pathway regulated by tumor suppressive miRNAs. **(15,17,19)**

· miR-1/133a cluster regulates actin cytoskeletal pathway

· miR-29a and miR-218 regulates laminin -integrin pathway

· miR-874 targets histone deacetylase pathway.

· miR-21 regulates Ras/MEK/ERK/PTEN/PDCD4 pathways

· miR-221 and miR-222 regulates AKT pathway

· miR-155 regulates TGF beta/smad pathway

· miR-26a/b regulates TMEM184B

· miR-31 regulates FIH-HIF-EVGF pathway

· miR-7 regulates RECK pathway

· miR-155 regulates CDC73

· miR-218 regulates Wnt/B- Catenin pathway.

H: miRNAs in oral cancer pain research: Hypo expression of miRNAs was demonstrated in the trigeminal ganglion neurons of an experimental model of inflammatory myogenic pain and it was demonstrated that miRNA act at the peripheral nervous system and that changes in their expression can be related to the development of both allodynia and hyperalgesia.

The cytokines like prostaglandin E2 (PGE2), interleukin 1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) can affect the expression of miRNAs and were also modulated by miRNA.

It was also suggested that, some promising miRNAs such as miR-125b-3p, miR-125b-5p, miR-181, miR-30d-5p, miR-379-5p and miR-339 have been correlated to pain. It is also reported that, the over expression of miR-181a and miR-181b may increase lymph node metastasis, vascular invasion by tumor and poor prognosis in Oral squamous cell carcinoma patients, suggesting that it can be a potential biomarker of the disease. (1)

Future implications of miRNA:

· Field of miRNA is growing rapidly and an emphasis on extra cellular miRNA expression is though established, limited studies are there on salivary miRNA and no studies on urinary miRNAs as diagnostic and prognostic biomarkers in OSCC.

· miRNAs may in future be administered in cancer therapeutics either as single agent or in combination therapies, making miRNA –targeted therapy in clinical trials more feasible.

· New methods to study miRNA have used coimmunoprecipitation of Ago proteins followed by micro array analysis of bound mRNA as a means to identify direct targets of miRNAs. (20)

Conclusion

The field of epigenetics in general and miRNAs in particular is swiftly expanding. It continues to leave a huge impact in cancer diagnostics in head and neck region by its unique properties in terms of detection and regulation. miRNA imprints and its signature profiles in oral cancer merits investigation as they also maintain specificity and sensitivity as an archetypal biomarker.

Conflict of Interest: No Conflict of interest

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