ADVANCEMENTS IN NON-INVASIVE ORAL CANCER DETECTION: UNVEILING INSIGHTS FROM CIRCULATING MIRNA PROFILING

Abstract

Within the pages of this book chapter lies an in-depth exploration of the present status of circulating miRNAs, illuminating their promising role in the realm of oral cancer detection. These microRNAs (miRNAs), small yet powerful non-coding RNA molecules, are pivotal in orchestrating gene expression regulation. Recent research breakthroughs have unwrapped their potential as biomarkers across various diseases, with oral cancer being no exception. This chapter delves into the profound significance of miRNAs, unraveling the methodologies employed in their detection, and shedding light on their distinct expression profiles as they relate to oral cancer.

Moreover, this chapter embarks on an insightful journey through the potential diagnostic and prognostic value held by miRNAs in the context of oral cancer. Beyond the promising aspects, the challenges entwined with integrating circulating miRNAs into clinical practice find their place in these discussions. Looking forward, the book chapter aims to guide the reader through the potential pathways that the utilization of circulating miRNAs might follow, thus shaping the future landscape of oral cancer detection and patient care.

Keywords: Medical sciences, cancer, Biopsies.

Authors

Dr. R. Poongodi
Assistant Professor
Pathology
AIIMS
Mangalagiri, Andhra Pradesh, India.

Dr. S. Arundhathi
Additional Professor
Pathology
AIIMS
Mangalagiri, Andhra Pradesh, India.

Dr. R. Sudhakar
Associate Professor
Pathology
NRI Medical college,
Chinakakani, Andhra Pradesh, India.

I. SHAPING THE FUTURE OF OSCC DIAGNOSIS: LIQUID BIOPSIES AND BEYOND

"While oral squamous cell carcinoma (OSCC) remains the second most prevalent cancer in India, its diagnosis primarily relies on visual inspection, cytological analysis, and biopsies followed by histopathological examinations. However, obtaining accurate biopsies is challenging due to sampling issues, difficulty in distinguishing cancer from reactive tissue, and complications with hard-to-reach tumors, often leading to multiple biopsies. This elongates the diagnostic process, allowing cancer to potentially spread. Shockingly, many oral cancer cases are diagnosed in advanced stages, contributing to high 5-year mortality rates.

To address these challenges and reduce mortality, improved diagnostic methods are crucial. A non-invasive tool is urgently needed to predict and differentiate cancer from benign lesions. Liquid biopsies, based on molecular techniques, have emerged as a promising approach for non-invasive cancer detection.

Evolution of Liquid Biopsies: Advancements in molecular techniques have reshaped the landscape of cancer diagnostics, shifting towards non-invasive methods. Liquid biopsies, centered around the analysis of circulating biomolecules in bodily fluids, have emerged as a promising avenue for early cancer detection and monitoring. The rationale behind liquid biopsies lies in the fact that tumors shed various components, such as DNA, RNA, and proteins, into the bloodstream. Among these, microRNAs (miRNAs) have garnered considerable attention due to their stability in circulation, distinct expression patterns in different disease states, and potential functional roles in tumorigenesis.

Liquid biopsies leverage bodily fluids—such as blood, serum, plasma, saliva, cell-free DNA, RNA, and microRNA—as analytical samples. Of particular interest are microRNAs, small non-coding RNAs frequently perturbed in cancers. Given their role in cancer pathways, stability for analysis, diagnostic potential, and therapeutic prospects, microRNAs present a promising role for non-invasive oral cancer detection.

II. MIRNAS AS PROMISING BIOMARKERS

MicroRNAs (miRNAs) are non-coding small RNA molecules that play a pivotal role in post-transcriptional gene regulation. Despite their size, miRNAs wield significant influence over expression of genes by binding to target messenger RNA (mRNA) molecules, leading to their degradation or translational inhibition. This regulatory mechanism affects a wide array of biological processes, including development, differentiation, and disease progression.

In the context of cancer, miRNAs have gained substantial attention as potential diagnostic and prognostic markers, as well as therapeutic targets. Dysregulated miRNA expression profiles are a hallmark of many cancer types, contributing to tumorigenesis and disease progression. Their stability in bodily fluids, such as blood, serum, and saliva, has led to their emergence as non-invasive biomarkers for cancer detection and monitoring through liquid biopsies.

MiRNAs' ability to reflect the complex molecular landscape of tumors holds promise for personalized medicine, aiding in treatment selection and response prediction. As the understanding of miRNA's intricate roles continues to evolve, these small molecules stand as a bridge between fundamental molecular biology and cutting-edge clinical applications, revolutionizing the field of cancer research and paving the way for innovative diagnostic and therapeutic strategies.

Diagnostic Potential of Circulating MiRNAs in Oral Cancer: Recent studies have explored the diagnostic potential of circulating miRNAs in oral cancer detection. These investigations have focused on identifying specific miRNA signatures that distinguish oral cancer patients from healthy individuals or those with benign lesions.

- **Methodologies for miRNA Detection:** The exploration of miRNA detection has witnessed the development of diverse techniques, each offering a unique set of advantages and considerations. These methodologies play a pivotal role in unraveling the intricate landscape of miRNA expression, contributing to the identification and validation of specific miRNA biomarkers.

  - **Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR):** This well-established technique is characterized by its accuracy and sensitivity. It allows for the quantification of miRNA levels by amplifying the target miRNA through reverse transcription followed by real-time PCR. qRT-PCR's reliability and suitability for analyzing specific miRNAs make it a cornerstone in miRNA research. Its quantitative nature enables the comparison of miRNA expression levels across different samples.

  - **Microarrays:** Microarray technology facilitates the high-throughput analysis of miRNA expression profiles. It involves the labeled miRNAs to hybridize with complementary probes immobilized on a chip. Microarrays offer a comprehensive view of miRNA expression patterns, enabling the simultaneous examination of numerous miRNAs. While microarrays offer valuable insights into miRNA profiles, they may be constrained by the specificity of probe design and require relatively large sample quantities.

  - **Next-Generation Sequencing (NGS):** NGS has revolutionized genomics and transcriptomics research, extending its impact to miRNA analysis. NGS enables the unbiased sequencing of miRNAs, facilitating the discovery of novel miRNAs and the determination of their expression levels. This technique offers unparalleled depth and resolution, uncovering the complexity of miRNA populations within a sample. However, NGS comes with computational challenges associated with data processing and analysis.

  - **Droplet Digital PCR (ddPCR):** ddPCR is a powerful technique that partitions a sample into numerous nanoliter-sized droplets, allowing for the absolute quantification of miRNA copies. This approach provides precise and reproducible quantification, offering advantages in detecting subtle miRNA expression changes. ddPCR's ability to accurately quantify low-abundance miRNAs makes it valuable for biomarker validation.

Each of these methodologies contributes uniquely to our understanding of miRNA expression dynamics. By harnessing the strengths of these techniques, researchers are able to pinpoint specific miRNA biomarkers with potential diagnostic, prognostic, and therapeutic implications. As the field continues to evolve, the integration of these techniques will undoubtedly pave the way for uncovering the intricacies of miRNA-mediated regulation in oral malignancies and other diseases.

- **Circulating miRNAs:** A Window into Diagnostic and Prognostic Landscapes

  Circulating miRNAs present several compelling attributes as markers for diagnosis and prognosis. They exhibit stability within bodily fluids, offer the convenience of non-invasive collection, and hold the ability to reflect disease status. Extensive research has unveiled differentially expressed miRNAs miRNAs in the bloodstream of individuals with oral cancer when contrasted with those who are healthy. These profiles carry the potential to facilitate early detection, enable risk stratification, and enhance the monitoring of treatment responses. Multiple investigations have pinpointed miRNAs with altered expression in oral cancer tissues, and these miRNAs are intricately involved in fundamental processes such as cell proliferation, apoptosis, invasion, and metastasis. The exploration of both tissue-specific and circulating miRNAs has forged pathways towards harnessing their prospective applications in diagnostic and prognostic contexts.

- **Upregulated Circulating miRNAs in Oral Cancer:** Diagnostic Potential

  Many studies have highlighted specific miRNAs that are significantly upregulated in the circulation of oral cancer patients. For instance, miR-21, a well-established oncomiR, has been consistently found to be elevated in oral cancer patients' blood samples. miR-31, miR-186, and miR-375 have also demonstrated upregulation, implicating their potential as diagnostic biomarkers. These upregulated miRNAs often target genes involved in cell cycle regulation, apoptosis, and metastasis, contributing to oral cancer pathogenesis.

- **Downregulated Circulating miRNAs in Oral Cancer:** Diagnostic Insights

  Conversely, certain miRNAs exhibit downregulation in the circulation of oral cancer patients. miR-125a-5p, known for its tumor-suppressive role, is frequently decreased in oral cancer patients. Similarly, miR-145 and miR-133a are frequently downregulated, contributing to enhanced cell proliferation and invasion. The downregulation of these miRNAs may release their inhibitory effects on oncogenes, further driving oral cancer progression.

- **Promise of Personalized Treatment and Prognostication:** Looking beyond the realm of diagnosis, the potential of circulating miRNAs stretches toward shaping personalized treatment strategies and refining prognostication in oral cancer. The inherent capacity of miRNAs to mirror the intricate molecular landscape of tumors within the bloodstream introduces the prospect of tailoring treatment approaches based on individualized miRNA profiles.

This groundbreaking approach could usher in a new era of precision medicine, where therapeutic interventions are finely tuned to a patient's unique biological characteristics.

Moreover, the miRNA signatures encapsulate invaluable insights into the trajectory of disease progression. By deciphering these molecular clues, more precise prognostic information can be harnessed, thereby enhancing the ability to anticipate the course of the disease and tailor interventions accordingly. This potential for refined prognosis holds the potential to revolutionize patient management and decision-making.

In a broader context, envisioning the integration of miRNA biomarker panels opens doors to an entirely new paradigm in OSCC screening, early detection, and continuous monitoring. By capitalizing on the multifaceted information carried by miRNAs, a comprehensive approach can be envisaged that not only aids in identifying the disease but also in navigating its dynamic course. The implications are far-reaching, with the potential to significantly alleviate the burden of morbidity and mortality associated with oral cancer.

As this field advances, the personalized treatment and prognostic potential of circulating miRNAs places it at the forefront of revolutionary molecular advancements in cancer diagnosis and treatment. These transformative potential positions circulating miRNAs as central players in the ongoing pursuit of enhancing patient care and outcomes in the context of oral cancer.

III. CIRCULATING MIRNA LANDSCAPE IN ORAL CANCER: CROSSING BORDERS AND BRIDGING GAPS

The exploration of miRNA profiling within the context of oral squamous cell carcinoma (OSCC) has transcended geographical boundaries, resulting in a diverse array of studies that shed light on the role of these diminutive molecules in the realm of cancer. While initial investigations predominantly centered on tissue samples, recent undertakings have expanded their scope to encompass serum and plasma samples, recognizing the potential of circulating miRNAs as non-invasive biomarkers.

Interestingly, while certain studies have highlighted specific miRNAs or combinations thereof, a subset has delved into comprehensive genome-wide analyses, yielding profound insights into the landscape of miRNAs. A groundbreaking study led by Wong et al. in 2008 brought to the fore the altered expression patterns of miRNAs within the circulation of oral cancer cases, serving as a catalyst for the intensified exploration of circulating miRNAs in the domain of oral malignancies. Building on this foundation, Nakamura et al. identified a cluster of six serum miRNAs with potential for distinguishing OSCC patients from healthy individuals. Similarly, Lu et al. unveiled a panel of five miRNAs that demonstrated robust diagnostic efficacy. These investigations underscore the remarkable potential of miRNAs as discerning markers for oral malignancies.

Further amplifying our understanding, studies by Rabinowits et al. and Chang et al. have unveiled diverse miRNA profiles within the circulation. This expanded exploration not only enables the differentiation of cancer from benign lesions but also provides valuable insights into the intricate signaling pathways that orchestrate tumor progression.

In stark contrast, the landscape of miRNA profiling in OSCC within India remains relatively uncharted territory. Despite the wealth of tissue-based miRNA profiling studies that have illuminated dysregulated miRNAs in oral cancer, the comprehensive genome-wide analysis of serum/plasma-based miRNA profiles remains a lacuna waiting to be filled.

A recent study conducted by Mazumder and colleagues embarked on the levels of 24 miRNAs. Within their study of OSCC patients, five specific miRNAs, namely miR-483-5p, miR-31-5p, Let-7b-5p, miR-486-5p, and miR-30e-5p, exhibited significant elevations.Using an Elastic-Net model that incorporated four miRNAs, OSCC cases were classified from healthy controls with an impressive 80% sensitivity, 64.3% specificity, and an overall accuracy of 72.4%.Furthermore, it was observed that both miR-483-5p and miR-31-5p were significantly overexpressed in OSCC tissues. These miRNAs also exhibited significantly higher levels in the serum of patients with Leukoplakia and Verrucous carcinoma, suggesting their potential as valuable early disease markers. Additionally, the efforts of researchers like Pooja et al. have unearthed miRNAs that hold promise as diagnostic markers in the Indian context.

However, conspicuously absent is a holistic analysis that deciphers the genomic profile of circulating miRNAs in Indian OSCC patients. Acknowledging the vast potential of circulating miRNAs in realms such as early diagnosis, prognosis, and therapeutic stratification, the critical gap in understanding the circulating miRNA signature specific to Indian OSCC patients beckons to be bridged.

The dual perspectives of global and national research landscapes on circulating miRNAs in oral cancer converge to underscore the power of collaborative efforts. While international studies have laid the foundation, national endeavors, particularly within India, are positioned to illuminate unique insights into the context-specific intricacies of miRNA profiles in oral cancer patients. As these two realms intersect and inform each other, a more comprehensive and nuanced understanding of the role of circulating miRNAs in oral cancer emerges, holding promise for improving diagnostics, prognostics, and personalized therapies. The uncharted territories within both global and national contexts beckon researchers to continue their pursuit of unraveling the miRNA-mediated intricacies of oral cancer, offering potential breakthroughs that could transform patient care and outcomes.

IV. SIGNIFICANCE AND IMPLICATIONS OF THE CIRCULATING MIRNA OVER TISSUE MIRNA

With the promise of targeted therapies on the horizon, the need to outline the entire human miRNA genomic profile of OSCC is paramount. Notably, miRNA expression can vary across ethnic groups, necessitating ethnic-specific profiling to establish an appropriate biomarker panel.

While tissue-based analyses offer insights, they might not mirror the complex milieu of circulating miRNAs, which could herald personalized treatments. Future projects addressing this gap by conducting extensive miRNA profiling should be done to uncover novel miRNAs with implications for oral cancer.

V. CHALLENGES AND CONSIDERATIONS

The potential of circulating miRNAs in revolutionizing oral cancer diagnostics is undoubtedly promising, yet several intricate challenges necessitate careful consideration and proactive resolution. These challenges are crucial to ensure the successful translation of this innovative approach into practical clinical applications.

One pivotal challenge involves the standardization of isolation and detection methods for circulating miRNAs. As these miRNAs exist in diverse body fluids, such as serum, blood, and saliva, devising consistent and reliable protocols for their extraction is paramount. This standardization ensures that the quality and quantity of miRNAs extracted remain consistent across different samples, facilitating accurate and reproducible results. Variability in isolation methods can introduce noise and hinder the reliable identification of diagnostic miRNA signatures.

Another complex challenge pertains to the intrinsic heterogeneity of oral cancer itself. Tumors can exhibit diverse molecular profiles, even within the same type of cancer. This heterogeneity introduces a layer of complexity in identifying universal miRNA biomarkers. Overcoming this challenge requires extensive research to discern common patterns amidst the diversity, which can guide the development of broadly applicable miRNA-based diagnostic panels.

The establishment of robust bioinformatics pipelines for data analysis represents yet another significant challenge. The data generated from miRNA profiling techniques, such as next-generation sequencing, is voluminous and intricate. Developing computational tools that can accurately process, analyze, and interpret this data is imperative. These pipelines need to integrate various steps, including quality control, alignment, normalization, and statistical analysis, to distill meaningful information from the raw data. A streamlined and reliable bioinformatics approach is pivotal to ensure that miRNA biomarker discovery is both efficient and accurate.

Furthermore, the intricate interplay between miRNA expression patterns and confounding factors adds complexity to this field. Elements like age, gender, and lifestyle habits can influence miRNA expression. It is essential to factor in these variables to accurately distinguish cancer-specific miRNA changes from natural variations. Integrating demographic and clinical data into the analysis helps untangle the genuine disease-related alterations from other influences, enhancing the specificity and reliability of diagnostic miRNA signatures.

While the promise of circulating miRNAs in oral cancer diagnostics is inspiring, addressing the array of challenges is paramount. Standardizing isolation and detection methods, navigating cancer heterogeneity, building robust bioinformatics pipelines, and accounting for confounding factors collectively shape the foundation for reliable and

clinically applicable miRNA-based diagnostics. These challenges, when thoughtfully addressed, pave the way for the transformation of oral cancer detection and patient care.

VI. FUTURE DIRECTIONS

The evolving landscape of circulating miRNAs in oral cancer detection demands comprehensive longitudinal studies with larger patient cohorts to unlock their diagnostic potential. Integrating miRNA profiles with omics data, including genomics, proteomics, and metabolomics, offers enhanced precision in diagnosis and prediction. This multi-dimensional approach enables a holistic understanding of disease, refining risk stratification and treatment strategies. Novel computational algorithms and bioinformatics tools play a pivotal role in deciphering complex miRNA-mRNA interactions, unveiling regulatory networks, and identifying therapeutic targets. Collaborative efforts among interdisciplinary teams are crucial for harnessing circulating miRNAs' potential, ultimately translating findings into clinical applications. In conclusion, by prioritizing longitudinal studies, integrating omics approaches, and leveraging advanced computational methodologies, the future of circulating miRNA research in oral cancer detection holds promise for more accurate and personalized diagnostics, advancing oral cancer management and patient outcomes.

VII. CONCLUSION

In summary, the progress made in circulating miRNA profiling presents a pioneering strategy for addressing the complexities linked to oral cancer diagnosis, especially within India's context. The distinctive blend of non-invasiveness, precision, and the potential for personalized therapeutic interventions emphasizes the revolutionary essence of this methodology. As research in this realm continues to evolve, the synergy between global and domestic researchers through collaborative initiatives has the potential to significantly deepen our comprehension of the fundamental role that circulating miRNAs play in the realm of oral cancer. This collective endeavor not only enriches our insights into the disease's underlying mechanisms but also fuels the development of innovative diagnostic and therapeutic approaches that could substantially impact the landscape of oral cancer management and patient well-being.

REFERENCES

[1] Adeola H.A., Bello I.O., Aruleba R.T., Francisco N.M., Adekiya T.A., Adefuye A.O., Ikwegbue P.C., Musaigwa F. The practicality of the use of liquid biopsy in early diagnosis and treatment monitoring of oral cancer in resource-limited settings. *Cancers.* 2022

[2] Roi, A.; Boia, S.; Rusu, L.-C.;Roi, C.I.; Boia, E.R.; Rivis, , M. Circulating miRNA as a Biomarker in Oral Cancer Liquid Biopsy. Biomedicines 2023, 11, 965.

[3] Wong T, et al. (2008). Alteration of microRNA expression in plasma of patients with oral squamous cell carcinoma. Oral Oncology, 44(2), 167-173.

[4] Naito Y., Honda K. Liquid Biopsy for Oral Cancer Diagnosis: Recent Advances and Challenges. *J. Pers. Med.* 2023;13:303.

[5] Nakamura, K.; Hiyake, N.; Hamada, T.; Yokoyama, S.; Mori, K.; Yamashiro, K.; Beppu, M.; Sagara, Y.; Sagara, Y.; Sugiura, T. Circulating microRNA Panel as a Potential Novel Biomarker for Oral Squamous Cell Carcinoma Diagnosis. Cancers 2021, 13, 449.

[6] Nakamura S, et al. (2016). Clinical relevance of circulating cell-free microRNAs in plasma of patients with oral squamous cell carcinoma. Oral Oncology, 53, 96-101.

[7] Lu YC, et al. (2017). Oncogenic function and early detection potential of miRNA-10b in oral cancer as identified by microRNA profiling. Cancer Prevention Research, 10(2), 115-124.
[8] Rabinowits G, et al. (2014). Exosomal microRNAs as potential biomarkers of cancer. Journal of Cellular Biochemistry, 115(12), 2207-2211.
[9] Schneider C, et al. (2015). Identification of microRNA expression profiles in blood and tissues as potential biomarkers for early detection of oral squamous cell carcinoma. Gene, 558(2), 232-238.
[10] L. He, et al. (2019). Identification of differentially expressed miRNAs from the serum of patients with oral squamous cell carcinoma by small RNA sequencing. Oncology Letters, 18(4), 4161-4170.
[11] Mazumder S., Basu B., Ray J.G., Chatterjee R. MiRNAs as non-invasive biomarkers in the serum of Oral Squamous Cell Carcinoma (OSCC) and Oral Potentially Malignant Disorder (OPMD) patients. *Arch. Oral Biol.* 2023
[12] Pooja S, et al. (2019). The expression of microRNA-21 in circulation is significantly related with the differentiation and prognosis of oral squamous cell carcinoma. Oral Oncology, 96, 7-13.

