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Literature Review

MicroRNA-21 expression as a novel diagnostic and prognostic biomarker in oral cancer: A narrative review

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ABSTRACT

Background: Oral cancer is a life-threatening disease that has 377.713 new cases every year and 60% of the 5-year overall survival rate globally. Approximately 84–97% of oral cancer arises from squamous cells, categorized as oral squamous cell carcinoma (OSCC). MiR-21 is a single-stranded, non-coding RNA that has been studied for its role in carcinogenesis. Overexpression of miR-21 is found in various cancers and is linked to a poor prognosis. However, few studies analyze the expression of miR-21 as a diagnostic and prognostic biomarker in oral cancer. **Purpose:** This review aimed to describe the expression of miR-21 as a novel diagnostic and prognostic biomarker in oral cancer. **Review:** MiR-21 was found to be upregulated in various cancers, including oral cancer. miR-21 targets several tumor suppressors such as PTEM, TPM1, and PDCD4 to modulate characteristics linked to cancer prognosis, including cell proliferation, apoptosis, invasion, and metastasis. Furthermore, the constant increase of miR-21 expression in healthy oral mucosa to oral potentially malignant disease to OSCC demonstrated its diagnostic value. **Conclusion:** miRNA-21 may act as a novel diagnostic and prognostic biomarker of oral cancer.

Keywords: miRNA; non-communicable; medicine; cancer; oral cancer

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INTRODUCTION

Oral cancer is a neoplasm with a high mortality rate, despite scientific advances.[1] Oral squamous cell carcinoma (OSCC) is the term used to describe oral malignancies which are histologically derived from squamous cells. OSCC can develop in various parts of the oral cavity including the palate, pharynx, gingiva, tongue, floor of the mouth, and buccal mucosa.2 Globally, there are approximately 377.000 new cases of OSCC diagnosed every year, with a mortality rate under 2% in all regions. In addition, it was found that more than 177.757 people were victims of OSCC in 2020, indicating oral cancer as one of the top 20 malignancies.[1,2] The most prevalent oral cancer (84–97%) is oral squamous cell carcinoma (OSCC).[3]

Oral carcinogenesis is a result of several processes initiated by numerous discrete genetic mutations. Molecular alterations, caused by exogenous (smoking, alcohol, etc.) and endogenous (syndrome, genetic predisposition, etc.) predisposing factors, gradually accumulate in the process. These modifications together initiate the transition from healthy cells to cancer cells, both microscopically and clinically.[4] 80% of oral cancer cases are associated with tobacco use and alcohol consumption, indicating that tobacco and alcohol are the main causes of oral cancer.[5] Smoking increases the chance of OSCC formation by 79%, and drinking more than 50 grams of alcohol each day increases the risk by 69%.[6] Other risk factors are known to be associated with other pathological conditions, such as precancerous lesions (e.g., oral lichen planus, submucous fibrosis, and leukoplakia) and infectious agents (e.g., human papillomavirus, Epstein-Barr virus, and hepatitis C virus). Additionally, the etiology of oral cancer may be influenced by traumatic events, nutritional variables, genetic factors, ultaviolet (UV) radiation, and immunosuppression.[5]

The gold standard for oral cancer diagnosis is a biopsy.[6] However, saliva can also provide critical

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pieces of information for diagnosis because it contains Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) molecules, cytokines, extracellular vesicles, and derivative tissues.[7] These molecules might be useful to diagnose oral cancer early. Numerous tumor-suppressive and oncogenic microRNAs are found to be involved in carcinogenesis. Micro Ribonucleic Acid (miRNA) is a small single-stranded non-coding RNA consisting of approximately 18–22 nucleotides, which plays an important role in gene expression regulation.[8] Studying the underlying mechanism of miRNA involvement in carcinogenesis can develop a valuable tool for the diagnosis, prognosis, and therapy of cancer.

Information regarding the prognostic value of miR-21 can determine prognosis at the time of diagnosis and convey treatment through therapeutic and surgical approaches with low or high levels of aggressiveness. Practicians will utilize the knowledge gained from individualized prognostic factors to design a specific treatment for the patient.[5] Apart from prognostic factors, miRNAs can also be used as targets for therapy, either by downregulating oncogenic or upregulating tumor-suppressive miRNAs. Further research is needed to determine if this strategy is more beneficial as a monotherapy or adjuvant therapy.[1] The aim of this review is to describe the expression of miR-21 as a novel diagnostic and prognostic biomarker in oral cancer.

REVIEW

This article is based on research and literature published from January 2012 until December 2022, which was selected by extracting data from Pubmed, ScienceDirect, and Google Scholar using the keywords: "microRNA-21" OR "miRNA-21" OR "miR-21") AND ("oral cancer" OR "oral squamous cell carcinoma" OR "OSCC") AND ("diagnostic" OR "prognostic") AND ("biomarker" OR "marker"). Articles were also searched manually in case relevant publications might have been missed due to saturation. All original full-text English articles were retrieved, and then the bibliographies were manually cross-checked for other relevant articles.

Oral squamous cell carcinoma (OSCC) has a poor prognosis, as depicted by 60% of the 5-year overall survival rate. Although several characteristics in cases, such as lymph node metastasis, perineural invasion, and severe stages, are associated with a poor prognosis, there is no definite way to identify them clinically. This lack of predictive markers attributed risk to oral cancer recurrence.[9] MicroRNAs (miRNAs) are single-stranded, non-coding RNAs that consist of approximately 19–24 nucleotides and regulate numerous downstream targets. miRNAs play an important role in many cellular functions, including proliferation, differentiation, and apoptosis.[10] miR-21 is one of the miRNAs that has been studied for its role in multiple cancers.[11] Clinically, a worse prognosis is linked to higher miR-21 expression in various cancers, such as breast cancer, lung cancer, and colon cancer, including oral squamous cell carcinoma.[10] It is found that miR-21 targets various tumor suppressors, including PTEN, TPM1, and PDCD4.[11] miR-21 expression is also found to be steadily increased in the progression of normal mucosa to leukoplakia to OSCC.[12]

DISCUSSION

MiR-21 expression was found to be upregulated in oral cancers. Zheng *et al.* compared miR-21 expression by immunohistochemical staining in OSCC and normal tissues. MiR-21 was reported in 80% of OSCC tissues and found to be lower in the early stages than in the advanced stages.[13] Similarly, Priya *et al.* also reported that miR-21 was overexpressed in OSCC tissues compared to its endogenous control through real-time polymerase chain reaction (RT-PCR).[14]

MiR-21 targets various tumor suppressor genes, including phosphatase and tensin homolog deleted on chromosome 10 (PTEN). Downregulation of PTEN in cancer is associated with high cell proliferation, invasion, and migration. It is reported that miR-21 was found to directly target PTEN. Suppression of miR-21 expression inhibits OSCC cell proliferation and promotes apoptosis.[13] In breast cancer, miR-21 was found to also target PTEN and modulate the invasiveness of cancer cells via the AKT and ERK1/2 pathways.[15]

Another target of miR-21 is tropomyosin-1 (TPM1), a cytoskeleton involved in muscle contraction. In carcinogenesis, TPM1 is found to function as a tumor suppressor. TPM1 expression is inhibited in many cancers, including OSCC. Since miR-21 might be involved in OSCC cell metastasis, it is possible that miR-21 might modulate the expression of TPM1. OSCC cells with downregulated TPM1 are found to have high invasion and metastasis abilities.[16]

Hedbäck *et al.* found that patients with high expression of miR-21 have shorter disease-free survival and vice versa. In OSCC cases, stromal myofibroblast cells and vessels were the ones that expressed high miR-21 levels rather than tumor cells. However, the reasoning behind this phenomenon is yet to be understood. Furthermore, high levels of stromal myofibroblast in cancer cells are often correlated with high mortality.[9] Myofibroblast is a modified fibroblast with high myofilaments, a prominent and rough endoplasmic reticulum, and collagen-producing Golgi apparatus. Myofibroblasts are found in oral cancer and wounds and are characterized by the presence of α-smooth muscle actin.[17,18] Myofibroblasts support tumor progression by modulating cytokine stimulation and maintaining the vascularization of cancer cells.[19] Thus, it is suggested that myofibroblasts have the ability to remodel connective tissue and promote angiogenesis, supported by findings in highly invasive breast cancer that have abundant myofibroblasts and higher microvessel density.[18]

Hedbäck *et al.* also demonstrated that tumor location, whether in the tongue or floor of the mouth, has no

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discernible influence on the expression of miR-21 in OSCC. Elevated expression of stromal miR-21 may be utilized as a stand-alone predictive biomarker (hazard ratio of 2.7) for OSCC disease-free survival.[9] Arantes *et al.* also found overexpression of miR-21 to be correlated with poor treatment responses to organ preservation protocols and worse survival. 66.7% of patients who showed incomplete responses to chemoradiation were found to have higher expression of miR-21 compared to those who responded. miR-21 is then concluded to be valuable as an independent prognostic factor (hazard ratio of 2.05).[20]

Gombos *et al.* also reported that miR-21 expression is upregulated in OSCC cells. Furthermore, it has a high tumor-to-normal ratio. They discovered a considerable overexpression of miR-21 after comparing the expression using a paired test, and the receiver operating characteristic (ROC) curve revealed a sensitivity and specificity of above 90%. This indicates miR-21 has the potential to be a diagnostic biomarker for OSCC.[11]

miR-21 expression is upregulated consistently from normal mucosa to oral leukoplakia (OLP) to OSCC.[12] Similarly, Brito *et al.* found miR-21 expression to be increased in oral leukoplakia that progresses to OSCC. It demonstrates miR-21 is not only an indication of OLP severity but also might be involved in tumor initiation.[21] In these cases, miR-21 is found to suppress programmed cell death-4 (PDCD4), a tumor suppressor involved in apoptosis.[11,22] PDCD4 can inhibit cell transformation into malignancy and is found to be downregulated in oral cancers. In colorectal cancers, miR-21 was also found to downregulate PDCD4 via the target site in the 3′-UTR at the posttranscriptional level. Thus, it further explains how miR-21 might be useful as an OSCC diagnostic biomarker.[15]

Kawakita *et al.* revealed that miR-21 is overexpressed in oral tongue squamous cell carcinoma. Furthermore, miR-21 increased tongue squamous carcinoma cell invasion via the Wnt/β-catenin pathway by targeting Dickkopf WNT Signaling Pathway Inhibitor 2 (DKK2). DKK2 expression is downregulated in tongue cancer and functions as an inhibitor of the Wnt/β-catenin signaling pathway.[15] Low expression of DKK2 is also reported to suppress proliferation and invasion in prostate cancer cells.[23]

Studies have demonstrated that miR-21 is an independent biomarker of poor prognosis that is substantially overexpressed in oral carcinomas and is associated with tumor development and survival. However, Supic *et al.* found that patients with overexpressed miR-21 only demonstrate a tendency to have poor overall disease survival without significant correlation to clinicopathological data. These results might be explained by the study's relatively small sample size. Alternatively, because it has been noticed in leukoplakia, an oral premalignant lesion, overexpression of miR-21 may be involved in the initiation of oral carcinogenesis.[24] From this narrative review can be concluded that miRNA-21 may act as a novel diagnostic and prognostic biomarker of oral cancer.

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