European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 11 , 2020

Micro RNA as a potential biomarker in Oral Leukoplakia – A Review

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Abstract : Oral squamous cell carcinoma (OSCC) is mostly diagnosed clinically in later stages and might be transformed by oral leukoplakia (OL) which is one of the potentially malignant disorders, presented as white patches or plaque. The local recurrence (up to 30%) and the percentage of dysplasia or transformation to malignancy is around 15.6% and 39.2%. Few lesions without dysplastic and mild dysplastic lesions also might turn out to be malignant. Consequently, only histopathologic investigation is not sufficient for scrutinizing leukoplakia which might turn to OSCC, particularly when these lesions do not show dysplastic characters in histopathologic observation. In the current era of molecular biology, the miRNA is the emerging key performer and is creating a revolution in carcinogenesis. Focusing on identifying the importance in existing studies to suggest areas for future research on miRNA as a potential target in the detection of early malignancy in leukoplakia is needed. This review intended to recognize possible microRNAs (miRNAs) for the early identification of oral cancer and its pathophysiology.

Keywords: Biomarker, micro RNA, oral leukoplakia, oral cancer.

Introduction

In regular practice, oral white lesions are detected commonly by health care professionals. Most of them being benign or some keratotic and inflammatory mainly investigated histopathologically for the presence of dysplastic features or malignancy.[1] Oral Leukoplakia is one of the potentially malignant disorders, presented as white patches or plaque. The local recurrence (up to 30%)[2] and the percentage of dysplasia or transformation to malignancy is around 15.6% and 39.2%.[3-5] Histologically 80% of leukoplakia diagnosed as non-dysplastic or low-grade dysplastic lesions, cases showing epithelial hyperplasia and/or hyperkeratosis are usually under repeated recall for observation'.[6] High-grade and moderate epithelial dysplastic cases (17%) diagnosed in leukoplakia are surgically treated.[7] But on the contrary few lesions without dysplastic and mild dysplastic lesions also might turn out to be malignant.[6-14] Consequently only histopathologic investigation is not sufficient for scrutinizing leukoplakia which might turn to OSCC, particularly when these lesions do not show dysplastic characters in histopathologic observation.

Now a day's focus is driving towards the management of potentially malignant lesions and cancer regulation from microRNA (miRNA). In humans, the expression of miRNAs has already been proved to influence the pathogenesis of certain benign and malignant oral conditions. In the current era of molecular biology, the miRNA is the emerging key performer and is creating a revolution in carcinogenesis.[15-17] The studies on the expression of miRNAs in tissue samples, saliva, blood, and cell lines were included as a biomarker for leukoplakia. Focusing on identifying the importance in existing studies to suggest areas for future research on miRNA as a potential target in the detection of early malignancy in leukoplakia is needed.[18]

Therefore, recognition of such biomolecules which could be utilized as a therapeutic tool for these oral diseases can be of real benefit. This review emphasizes the widely concealed and unevaluated topic: miRNAs as a potential biomarker in oral leukoplakia and their possible role in pathophysiology.

MicroRNA biogenesis and function

MicroRNAs hold the asset of tiny non-coding RNAs made of 19–25 nucleotides. These noncoding RNAs play role in several biologic activities by regulating their specified genes as development, differentiation, apoptosis, and proliferation.[19-21] They negatively control gene expression at the post-transcriptional level. Genome-wide identification and calculative projection for targets of miRNA approximate one miRNA will bond to a number of more mRNAs, which all together turns for controlling of at least half of the protein-coding human genes.[22] Demonstrating the significance in their cell biology, should not be astonished about deregulation of miRNA is connected with numerous human diseases, even cancer.[23] The

biogenesis of miRNA is initiated within the nucleus and winds up within the cytoplasm. The inception of primary miRNA transcripts (pri-miRNA) normally synthesized from RNA polymerase II by 5′ cap and 3′ poly-A tail being the first step.[24] Numerous research is done in the field of cancer biology and miRNAs by managing the presence of targeted messenger RNAs (mRNAs) regarding the growth of a tumor, invading, angiogenic, and evading immune system in the past decades.[25,26] In the growth series of oral cancer, specific genes gain a part in tumorogenesis some of them being tumor suppressors. The elevated state of specific miRNAs is involved in the advancement of cancer and some of in suppressing it. Dysregulation of more than one-fourth of miRNAs is markedly detected for the minimum one cancer variant, predicting that miRNAs to be one amongst the biggest group of cancer gene regulators associated activities.[27] Currently, a group of researchers has proved about miRNAs can serve as cancer 'drivers' so even abnormal remarks would extremely commit for advancement from premalignancy to malignancy.[18,28] Besides the clinical presentation and histological features, the predilection to depict which leukoplakia will progress are difficult to assess.

Altered miRNA profiles in oral leukoplakia

Oral leukoplakia (OLK) clinically is considered to be the major types of oral potentially malignant disorder which exhibits as nonscratchable white lesion found on the oral mucosal membrane. Which has been linked with tobacco, alcohol, HPV infection, and genetic and epigenetic predisposition in the list of risk factors.[29] The origin and growth of OSCC are known to be a multifactorial task initiating from hyperplasia, proceeding to dysplasia, and ultimatum to neoplasia. In course of these events, many genetic variations can be observed, comprising mutation, amplification, or deletions in DNA, aberrations in chromosomes. Looking at the above events oral leukoplakia is identified as an eminent prototype to study oral carcinogenesis. It is also observed that certain OLKs do not respond to therapy and have recurrent up to 30% or may show transformation to malignancy. [2,3]

Many studies have recognized the expression of aberrant miRNA profiles in leukoplakia. These expression levels of miRNAs studied correlate clinicopathologically and show diagnostic and prognostic importance.[30] Table I enlists the deregulated miRNAs in oral leukoplakia from the various studies carried out. Several miRNAs expressed by these studies show a major biological role in tumor-suppressing or promoters of tumor for the inception and progressing of oral carcinoma.[31]

The miRNAs miR-21, miR-181b, and miR-345 have been overexpressed in some studies from which it can be imparted that this can be a biomarker in malignant transformation cases of oral premalignant lesions, leukoplakia, where histopathology has minimal predictive value. The overexpression of miR-21 has a role in the proliferation of cells and apoptotic property.[32] In a study conducted, on miR-21, miR-181b, and miR-345 upregulation was involved with an

aggressive oral progressive leukoplakia, in comparison with or without progressive leukoplakia.[33] Genes involved in tumor suppression, namely tropomyosin 1 (TPM1) and serpin peptidase inhibitor, clade B (ovalbumin), member 5 (SERPINB5), are reported to be in the focus of miR-21, suggesting it to be major for invading and metastasis of the tumor.[34] The miR-181b is seen in down-regulating the homeobox protein, HOXA11 (inhibitor of differentiation).[35] The miR-181 family has a direct role with of cell cycle progression and survival through controlling genes bcl-2.[36] MicroRNA 345, which is sensitive to methylation is known for proliferating and invading cells in human colorectal cancer which is even found in OLK.[37]

The observation in the studies with miR-196a and miR-206 was under-expression in premalignant lesions and overexpression in malignancies. The above observations might indicate the feature of these two miRs in dysplasias of mild to moderate which are progressive with under-expression, and their consecutive over-expression in severe dysplasias and invasive type of malignancy which may intricate in advanced stages of progression of OSCC.[33] This will provide a path to identify which microRNA is involved in the initiation of OSCC from leukoplakia.[38] Few studies revealed the miR-31 to be overexpressed or upregulated in OLP. This was the first miRNA to show altered expression in oral cancer. Ultimately, miR-31* is obtained by the prototype similar to miR-31 and it is seen to be overexpressed in the studied cases of mtOLK . In the squamous cell carcinoma seen in the head and neck, this particular MiR is proved to be oncogenic. The characteristic feature of miR-31* to harmonize apoptotic activity and cell movement might be enough to avert the recurrence of OLK and even initiation of leukoplakia. Functional investigation of miR-31* could expand knowledge regarding the pathogenesis of miRNA* components, particularly which are expressed together and paired miRNAs.[38,39] Even some studies showed upregulation of miR-31 and its inverse relation with the progression of leukoplakia. [33,40]

The miR-29a, miR-34b, and miR-423 were differentially expressed in a study conducted on leukoplakia compared to “control” tissues. These observations suggested that mir29a, mir34b, mir423, and Xpo5 expression was altered in leukoplakia which may imply the transformation of leukoplakia from the normal epithelium.[41] The miR-208-3p has a speculative oncogenicity and overexpression of it can intensify proliferation of cells, progression of the cell cycle, and tumorigenicity in esophageal squamous cells.[42] The miR-208 directly targets SOX6 a tumor suppressor gene and hence, overexpression of it causes downregulation of protein SOX6, in turn causing p21 downregulation, cyclin D1 upregulation, and Rb deregulation by phosphorylation.[42] Even the miR-3065-5p is anticipated to be tumor-suppressive, imparting in depletion migration of cells and invasion of tissue.[43] The miR-129-2-3p an oncogene expressed in OLP is a negative regulator of SOX4.[44] But hyper methylation of its promoter region which is followed by gene silencing of miR-129-2-3p showing overexpression of SOX4 and Cdk6 is eventually noted with tumorigenesis. The miR-204-5p is believed as a tumor suppressor and its downregulation by hypermethylation is found in increased metastatic activity and overall survival is decreased.[45]

The upregulation of miR-146a is documented with the factor NF-kB (nuclear factor-kB) which is the sign of path in OSCC cells.[46] This factor is an important gene regulator and transcriptional factor family which play an important part in the immune system, inflammation, stress reaction, apoptosis, and initiation of tumor in many malignant tumors. The miR-1269a is up-regulated by the expression of TGF-b1 in malignant cells, indicating TGF-b1 as an important entity that regulates miR-1269a.[47] Research conducted on TNF-a and TGF-b1 has shown that they stimulate great elevation in MMP-2 and MMP-9 and even in presence of protein in leukoplakia and OSCC.[48-50] The reduced risk of OSCC was correlated with the type G/G genotype of miR-146a C > G. Furthermore, one amongst two C allelic variant or C/C genotype of miR-1269b was seen less possible in BQ-related (betel quid) OLPs and BQ-related oropharyngeal squamous cell carcinoma. To conclude, the miR-146a and miR- 1269b with variant genotypes may be categorized as genetic markers in case of the growth of OPLs and head and neck OSCC.[51]

The research on the level of expression in serum miR-9 was seen that significant down-regulation of it in patients with OSCC or OLK, reporting miR-9 might be associated in the regulation of initiating and progressing of OSCC. Through the downregulation of the expression of CXC chemokine receptor 4 via the Wnt/b-catenin signaling way, the miR-9 might have a tumor-suppressive role.[52] The tumor-related gene NF-kappaB1was identified as a downstream targeted gene of miR-9 in stomach cancer.[53] The intensity of serum miR-9 was downregulated in cases of OLK and those in OSCC. Lower serum magnitude of miR-9 was involved in an advanced stage and the poor prognosis of OSCC. Collectively, according to all studies reviewed miR-9 demonstrated that it has a character of tumor suppression in OSCC and can be targeted as a possible therapeutic mode of treatment.[54]

The miR-26a and mir423 are found downregulated in both leukoplakia and malignancy indicating them as a tumor suppressor action in both entities. Currently, it is known that miR423 could aim at genes associated with apoptosis and even play role in autophagy.[55] Looking at these features, mir-423 might function as a tumor suppressor, in malignancy suggesting an alarming feature in leukoplakia. CDK6 has a binding locus for certain miRNAs but accepted results are there for miR-26a, miR-34b.[56,57] Cyclin-dependent kinases have a catalytic component the CDK6a which has the main place in the cell cycle of the G1 phase which is involved in various cancers including oral cancer.[58] Studies have already demonstrated CDK6 shows altered expression in OSCC and leukoplakia. The CPEB3 and PI3KR1 are confirmed targets of miR-29a and miR-26a and was observed that both these were under-expressed in leukoplakia and malignant tissues.[59] The miR-221/222 was down-regulated and has shown the property of promoting apoptosis in OSCC cells.[60] In non-malignant cases, miR-222-3p is been found in betel quid chewing, wherein miR-222-3p and miR-423-5p were shown in progressing of tumor and metastasis to a lymph node. These miRNAs are revealed in the involvement of pathways associated with cancer, as Wnt, PI3K-Akt, MAPK, and Ras.[61] The presence of miR-423-5p and

miR-150-5p in OLP is seen to be an oncomiR in course of the development of a tumor.[61] Widespread over-expression may be seen as the recognized miRs are found in genomic regions which are associated with cancer and maybe deregulated by the mechanism of epigenetics or modifications in the miR organizing tool. [62]

Conclusion

All these reviewed miRNA expressions denote the modifications of specific miRNAs and target genes which might show importance in progressing OPMD to malignancy. Expression portrait of miRNA and their genes will be helpful to identify suspicious leukoplakia from nonsuspicious type, hereby strengthening diagnostics aids. Disclosure of these predictive biomarkers which can precisely recognize histologically suspicious high-risk oral lesions in OSSC progression can appreciably play role in the improvement of the diagnostic outcome by mode of early intervention. Even the roles of miRNAs that are altered seem to have an important part in the initiating and progressiveness of OSCC by serving as oncogenes or as tumor suppressors. Regardless, it would be a big task further to interpret these favorable observations to clinicians before the following matter should be fully tackled.

Financial support: None

Conflicts of interest: None

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Table I Data extraction of Dysregulation of miRNAs associated with oral leukoplakia.

Sr No	Year, author, and population	Samples	Method	miR Dysregulated	
				Up Regulated	Down Regulated
1	2009, Nilva K. Cervigne et al, University of Toronto.	29 leukoplakia 14 carcinomas. Archival tissue.	RT- PCR	mir-146b mir-181b mir-21 mir-345 mir-518b mir-520g mir649 mir-184	mir-196a mir-206 miR-1, miR-133a and miR-133b
2	2011, Shanghai Jiao et al. Tong University.	20 leukoplakia 7 mtOLK cell lines Leuk-1, HIOEC, Cal-27,	RT-qPCR	mir-31	
3	2013 Yang et al. Shanghai Jiao Tong University.	45 leukoplakia Saliva samples	RT-qPCR	miR-708, miR-10b, miR-19a, miR-30e, miR-26a, miR-660	miR-99, miR-15a, miR-197, miR-145 miR-150.
4	2014, Navonil De Sarkar et al. Kolkata.	cancer 18 lichen planus 12 leukoplakia 18 Tissue	real-time PCR and TLDA	mir-31	mir-204
5	2014 Jo~ao A. R. Brito et al. Brazil.	22 leukoplakia 17 OSCC 6 normal tissue. Tissue samples	qPCR	miR-21 and miR-181b, miR-345	
6	2014, Roy et al. Kolkata.	225 leukoplakia Venous blood and biopsy tissue.	Real-Time PCR	mir29a, mir34b, mir423	
7	2016, Elizabeth Philipone et al.	100 leukoplakia formalin-fixed paraffin-embedded (FFPE) tissue	qRT-PCR	miR-129-2-3p and miR-204-5p , miR-208-5p	miR-3065-5p

	Columbia University Medical Center.				
8	2016, Hung-Chih Chen et al. Kaohsiung, Taiwan.	169 leukoplakia, 82 oral submucous fibrosis, Blood samples	Genotyping by TaqMan RT- PCR	miR-146a and miR- 1269b	
9	2016, Legang Sun et al. Hospital of Binzhou Medical College.	30 leukoplakia Blood samples (Serum),	Real-Time PCR		miR-9
10	2016, Roy et al , Kolkata.	20 leukoplakia, 20 lichen planus 20 cancer tissues. Tissues	qPCR assay		miR-26a and miR-29a
11	2018, Chang et al. Taiwan.	70 normal, 66 leukoplakia 114 OSCC Whole blood samples (plasma)	qRT-PCR and small RNA seq,	miR-423-5p and miR-150-5p	miR-222-3p