REVIEW

Molecular Markers of Precancerous Conditions of the Oral Mucosa

Kurbanova Patimat Arsenovna, Radzhabova Aminat Khiramagomedovna, Kuchaeva Salikhat Pashayevna, Aliyeva Arina Gadzhievna, Budaichiev Gasan Magomed-Alievich

Abstract

Introduction: Oral cancer is a significant public health issue, with a considerable portion of cases arising from precancerous conditions such as leukoplakia, erythroplakia, and oral submucous fibrosis. Early detection and timely intervention are critical for improving patient outcomes. This review explores the potential of molecular markers in the early diagnosis and management of these precancerous conditions of the oral mucosa.

Aim: The aim of this article is to compile and analyze current molecular markers used in diagnosing and predicting the progression of precancerous conditions of the oral mucosa. By understanding these markers, we aim to improve early detection and provide insights into more effective treatment strategies.

Materials and Methods: A comprehensive literature review was conducted using electronic databases such as PubMed, Google Scholar, and recent conference proceedings. The focus was on identifying key molecular markers and their clinical implications.

Results: The review highlights various molecular markers, including genomic, epigenetic, transcriptomic, and proteomic markers. Key markers discussed include TP53 mutations, miR-21, p16INK4a, Cyclin D1, and matrix metalloproteinases (MMPs), among others. These markers provide valuable information on the presence and degree of precancerous changes and the risk of their progression to cancer.

Conclusion: The integration of molecular markers into clinical practice can significantly improve the accuracy of early diagnosis and the effectiveness of treatment, thus enhancing patient prognosis. Further research is needed to standardize the use of these markers in routine clinical practice.

Keywords: Precancerous conditions, oral mucosa, molecular markers, biomarkers, early diagnosis

Introduction

Oral cancer continues to pose a significant public health challenge, characterized by high mortality rates and widespread prevalence. A crucial factor in understanding and combating this disease is recognizing that a considerable number of oral cancer cases originate from precancerous lesions. These lesions include conditions such as leukoplakia, erythroplakia, and oral submucous fibrosis. These precancerous conditions manifest as various alterations in the oral mucosa that, if not detected and managed in time, can progress to malignant neoplasms. Consequently, early detection and timely intervention are essential for improving patient prognosis and increasing the chances of complete recovery.[1]

The utilization of molecular markers has emerged as a promising pathway for the non-invasive and early diagnosis of precancerous conditions of the oral mucosa. Molecular markers encompass a range of genetic and epigenetic changes that occur at the cellular level during the early stages of pathological processes. These markers can be categorized into genomic, epigenetic, transcriptomic, and proteomic types. Genomic markers include mutations and loss of heterozygosity; epigenetic markers involve changes like DNA methylation and histone modifications; transcriptomic markers cover alterations in microRNA expression; and proteomic markers involve the expression of specific proteins. Each of these markers provides critical insights into the presence and extent of precancerous changes and the risk

Department and Institution Affiliation: Dagestan State Medical University, Makhachkala, Dagestan Republic, Russia

Corresponding Author: Budaichiev Gasan Magomed-Alievich, Dagestan State Medical University, Makhachkala, Dagestan Republic, Russia. E-mail: gasan.budachiev005@mail.ru

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of their progression to cancer.[2]

Incorporating molecular markers into clinical practice significantly enhances the accuracy and timeliness of diagnoses. This improvement enables more effective treatment and monitoring of patient conditions. Molecular markers facilitate screening and diagnosis at the molecular level, allowing for the detection of pathological changes long before clinical symptoms become apparent. This early detection capability empowers clinicians to intervene more effectively, potentially preventing or reversing the progression of precancerous conditions.[3]

This review aims to examine the various molecular markers identified in recent studies and explore their potential applications in clinical practice. By compiling and analyzing current research, this review seeks to underscore the importance of these markers in the early detection and management of precancerous conditions of the oral mucosa.

Precancerous conditions of the oral mucosa.

Leukoplakia: Leukoplakia is a white patch or plaque on the oral mucosa that cannot be wiped off and cannot be clinically or pathologically diagnosed as any other condition. It is one of the most common precancerous conditions in the oral cavity. The primary molecular marker of leukoplakia is mutations in the TP53 gene, which are often observed in various types of cancer, including oral cancer. TP53 mutations can indicate an increased risk of malignant transformation. The TP53 gene encodes a tumor suppressor protein that plays a critical role in regulating the cell cycle, promoting DNA repair, and initiating apoptosis in response to cellular stress or DNA damage. Mutations in TP53 can lead to the loss of these vital functions, resulting in uncontrolled cell proliferation and an increased potential for the development of malignancy. Other important markers in leukoplakia include miR-21 and p16INK4a. miR-21 is a microRNA whose overexpression is associated with the progression of cancer processes. MicroRNAs are small non-coding RNAs that regulate gene expression post-transcriptionally, typically by binding to the 3′ untranslated region of target mRNAs, leading to their degradation or inhibition of translation. Overexpression of miR-21 has been linked to the inhibition of tumor suppressor genes, thereby promoting oncogenesis. In the context of leukoplakia, elevated levels of miR-21 can enhance cell proliferation, invasion, and survival, contributing to the progression towards malignancy. p16INK4a, a product of the CDKN2A gene, plays a key role in cell cycle regulation by inhibiting cyclin-dependent kinases (CDKs) that are necessary for the transition from the G1 phase to the S phase of the cell cycle. Altered expression of p16INK4a is often observed in dysplastic lesions, including those in leukoplakia. The loss of p16INK4a function can lead to unchecked cell cycle progression and increased cellular proliferation, thereby elevating the risk of malignant transformation. The expression levels of these molecular markers can be assessed using various diagnostic techniques, providing valuable information for the early detection and management of precancerous conditions in the oral cavity.[4,5]

Erythroplakia: Erythroplakia is characterized by the presence of a red patch on the oral mucosa that cannot be clinically or pathologically diagnosed as any other condition. It is considered more dangerous than leukoplakia due to the high risk of malignant transformation. One of the key molecular markers of erythroplakia is the loss of heterozygosity at specific loci, which can contribute to the progression of precancerous conditions to cancer. Loss of heterozygosity involves the deletion or inactivation of one allele of a gene in which the other allele was already inactivated, leading to the loss of tumor suppressor functions and increased oncogenic potential. This genetic alteration is significant in the context of erythroplakia as it highlights the potential for malignant transformation. Another important marker is the overexpression of Cyclin D1, a protein that plays a critical role in cell cycle regulation and increased cell proliferation. Cyclin D1 is a regulatory protein that, when bound to its CDK partners, drives the cell cycle transition from the G1 phase to the S phase, promoting DNA synthesis and cell division. Elevated levels of Cyclin D1 are often observed in dysplastic and malignant lesions, indicating its role in the oncogenic process. Overexpression of Cyclin D1 can result from gene amplification, transcriptional upregulation, or post-transcriptional modifications, leading to increased cellular proliferation and a higher risk of progression to cancer. Assessing the levels of these molecular markers can provide critical insights into the malignant potential of erythroplakia, aiding in early diagnosis and targeted therapeutic strategies.[6,7]

Oral submucous fibrosis: Oral submucous fibrosis is a chronic, slowly progressing disease characterized by fibrosis of the oral mucosa, leading to significant tissue stiffness and restricted mouth opening. This condition is commonly associated with the chewing of betel quid, which is a major risk factor. A key aspect of oral submucous fibrosis is the elevated levels of transforming growth factor-beta (TGF-β), which plays a crucial role in the fibrotic process. TGF-β promotes the transformation of fibroblasts into myofibroblasts, which produce excessive amounts of extracellular matrix components, including collagen. This leads to abnormal collagen accumulation, contributing to the stiffness and reduced elasticity of the oral tissues. Altered expression of collagen-related genes, such as COL1A1 and COL1A2, is also observed in this condition. These genes encode for type I and type II collagen, respectively, and their overexpression results in excessive collagen deposition in the tissues. This overproduction of collagen further exacerbates the fibrotic process, leading to progressive tissue rigidity and functional impairment. Oral submucous fibrosis also involves dysregulation of matrix metalloproteinases (MMPs) and their tissue inhibitors, which are critical in maintaining the balance between collagen synthesis and degradation. The imbalance in this system favors collagen accumulation, contributing to the pathological changes seen in this condition. Overall, oral submucous fibrosis is marked by significant molecular changes, including elevated TGF-β levels and altered collagen gene expression, leading to excessive collagen accumulation and fibrosis. Understanding these molecular mechanisms is essential for developing effective diagnostic and therapeutic strategies to manage this debilitating condition.[8,9]

Genomic markers

TP53 Mutations: TP53 is a tumor suppressor gene that plays a critical role in preventing oncogenesis. It is often

referred to as the "guardian of the genome" due to its pivotal functions in maintaining cellular integrity. TP53 mutations are frequently observed in various cancers, including oral cancer, and can indicate an increased risk of malignant transformation of precancerous lesions into cancerous ones. The TP53 gene encodes a protein that regulates the cell cycle, promotes DNA repair, and induces apoptosis in response to DNA damage or cellular stress. When functioning normally, TP53 can halt the cell cycle to allow for DNA repair or trigger apoptosis if the damage is irreparable, thus preventing the propagation of genetic errors. However, mutations in TP53 can lead to a loss of these critical functions. Mutant TP53 proteins may lose their ability to bind DNA and activate transcription of target genes involved in cell cycle arrest and apoptosis. This loss of function leads to uncontrolled cell proliferation and tumor development, as cells with damaged DNA continue to divide and accumulate additional mutations. Furthermore, some TP53 mutations can result in a dominant-negative effect, where the mutant protein interferes with the function of the remaining wild-type protein, exacerbating the loss of tumor suppressor activity. In the context of oral precancerous lesions, the presence of TP53 mutations serves as a biomarker for increased malignancy risk, highlighting the importance of monitoring and potentially intervening in these lesions before they progress to full-blown cancer. The assessment of TP53 status in oral mucosal lesions can thus provide valuable prognostic information and guide clinical decision-making.[10,11]

CDKN2A/p16INK4a: The CDKN2A gene encodes the p16INK4a protein, which is an important regulator of the cell cycle. p16INK4a functions as a tumor suppressor by inhibiting cyclin-dependent kinases (CDKs) 4 and 6, which are essential for the transition from the G1 phase to the S phase of the cell cycle. By inhibiting these CDKs, p16INK4a prevents the phosphorylation of the retinoblastoma protein (Rb), thereby halting cell cycle progression and allowing for DNA repair or cellular senescence in response to oncogenic signals. Loss of function or altered expression of p16INK4a is often observed in dysplastic and precancerous lesions of the oral mucosa. These alterations can occur through various mechanisms, including gene deletions, promoter hypermethylation, or mutations that disrupt protein function. The absence or reduced expression of p16INK4a leads to unchecked CDK activity, resulting in uncontrolled cell proliferation and an increased risk of malignant transformation. This marker is important for assessing the risk of malignant transformation and progression of precancerous conditions. In clinical practice, the evaluation of p16INK4a expression can provide valuable insights into the biological behavior of oral lesions. Dysplastic lesions with low or absent p16INK4a expression are more likely to progress to oral squamous cell carcinoma (OSCC) compared to those with normal p16INK4a levels. Consequently, p16INK4a serves as a crucial biomarker for early detection and risk stratification of oral precancerous conditions, aiding in the timely implementation of preventive and therapeutic strategies.[12]

Epigenetic markers

DNA Methylation patterns: Hypermethylation of tumor suppressor genes, such as p16INK4a, and hypomethylation of oncogenes are frequently observed in precancerous and cancerous lesions. These epigenetic changes lead to alterations in gene expression, contributing to oncogenesis. DNA methylation involves the addition of a methyl group to the cytosine residues in CpG dinucleotides, typically resulting in the suppression of gene expression when occurring in gene promoter regions. In the context of oral precancerous lesions, hypermethylation of tumor suppressor genes like p16INK4a can lead to their silencing, which removes critical brakes on the cell cycle and allows for unchecked cellular proliferation. This epigenetic alteration is a key mechanism by which cells bypass normal growth control and progress towards malignancy. Conversely, hypomethylation of oncogenes can result in their overexpression, further driving the oncogenic process by promoting cell growth, division, and survival. DNA methylation is an important mechanism of gene regulation and can be used as a diagnostic and prognostic marker of precancerous conditions. The detection of specific methylation patterns in tissue samples can provide insights into the molecular changes underlying disease progression. For instance, the presence of hypermethylated p16INK4a in a biopsy sample may indicate a higher risk of malignant transformation, prompting closer monitoring and more aggressive management. Additionally, DNA methylation markers can be used to track the effectiveness of treatment and the potential for recurrence, making them valuable tools in personalized medicine and targeted therapy for oral precancerous conditions.[13,14]

Histone modifications: Changes in histone acetylation and methylation can reflect alterations in gene expression associated with oncogenesis. Histones are proteins around which DNA is wound, playing a key role in DNA packaging and gene transcription regulation. Post-translational modifications of histones, such as acetylation and methylation, can influence chromatin structure and accessibility, thereby regulating gene expression. Histone acetylation typically occurs at lysine residues and is associated with an open chromatin conformation that promotes active transcription. Acetylation neutralizes the positive charge on histones, reducing their affinity for the negatively charged DNA and allowing transcriptional machinery to access the DNA. Conversely, deacetylation by histone deacetylases (HDACs) leads to a closed chromatin conformation and gene repression. Histone methylation can have different effects on gene expression depending on the specific amino acid residues and the number of methyl groups added. For example, methylation of histone H3 at lysine 4 (H3K4) is generally associated with active transcription, while methylation at lysine 9 (H3K9) or lysine 27 (H3K27) is linked to gene repression. These histone modifications are crucial for the regulation of genes involved in cell cycle control, DNA repair, and apoptosis. In the context of precancerous conditions of the oral mucosa, aberrant histone modifications can lead to the inappropriate activation or silencing of genes that drive oncogenesis. For instance, hypoacetylation of tumor suppressor genes or hypermethylation of oncogenes can promote the progression from a precancerous state to malignancy. The assessment of histone modifications in biopsy samples can be used for the diagnosis and monitoring

of precancerous conditions. Techniques such as chromatin immunoprecipitation (ChIP) followed by sequencing (ChIP-seq) allow for the precise mapping of histone modifications across the genome, providing insights into the epigenetic landscape of oral lesions. Monitoring these changes can help in identifying high-risk lesions and tailoring treatment strategies to prevent cancer development.[15,16]

Transcriptomic markers

miR-21: miR-21 is a microRNA whose overexpression is associated with the progression of various cancers, including squamous cell carcinoma of the oral cavity. miR-21 functions by targeting and inhibiting tumor suppressor genes such as PTEN and PDCD4, which are involved in regulating cell proliferation, apoptosis, and invasion. By inhibiting these tumor suppressor genes, miR-21 promotes oncogenic processes, leading to increased cell proliferation and invasion, contributing to the malignant transformation of cells. The elevated expression of miR-21 in precancerous lesions makes it a useful biomarker for early diagnosis and monitoring of these conditions. Its detection in tissue or saliva samples can provide an early indication of malignant potential, allowing for timely intervention and management. Furthermore, monitoring changes in miR-21 levels over time can help assess the effectiveness of treatments and detect potential recurrences. Thus, miR-21 serves as a critical marker in the early diagnosis, prognosis, and therapeutic monitoring of precancerous conditions of the oral cavity.[17]

miR-34: miR-34a is often downregulated in cancer and acts as a tumor suppressor by regulating genes involved in the cell cycle and apoptosis. miR-34a exerts its tumor-suppressive effects by targeting multiple oncogenes and genes that promote cell proliferation, such as MYC and CDK6, thereby inhibiting uncontrolled cell growth and inducing programmed cell death. Reduced levels of miR-34a can indicate an increased risk of malignant transformation, as the loss of its regulatory function allows for the unchecked progression of cells through the cell cycle and evasion of apoptosis. This downregulation can be detected in precancerous lesions, making miR-34a a valuable prognostic marker. Monitoring miR-34a levels can help identify lesions with a higher likelihood of progressing to malignancy, enabling more aggressive surveillance and early therapeutic intervention. As a prognostic marker, miR-34a provides critical insights into the molecular changes occurring in precancerous conditions, aiding in risk assessment and personalized treatment planning.[18]

Proteomic markers

Cyclin D1: Overexpression of Cyclin D1 is associated with increased cell proliferation and is often observed in dysplastic lesions. Cyclin D1 plays a critical role in cell cycle regulation by promoting the transition from the G1 phase to the S phase. It does this by binding to and activating cyclin-dependent kinases (CDKs), which then phosphorylate target proteins to drive cell cycle progression. The overexpression of Cyclin D1 disrupts normal cell cycle control, leading to excessive cellular proliferation, a hallmark of dysplastic and precancerous conditions. Because of its critical role in cell cycle regulation, Cyclin D1 can be used as a diagnostic and prognostic marker of precancerous conditions. Elevated levels of Cyclin D1 in tissue samples can indicate the presence of dysplastic changes and an increased risk of progression to malignancy. Therefore, assessing Cyclin D1 expression can help in the early detection of precancerous lesions, guiding clinical decision-making and treatment planning. Additionally, monitoring Cyclin D1 levels can provide insights into the effectiveness of therapeutic interventions and the potential for disease recurrence.[19]

Matrix metalloproteinases (MMPs): Matrix metalloproteinases, such as MMP-9, are enzymes involved in the breakdown of the extracellular matrix, which facilitates cancer invasion and metastasis. These enzymes degrade various components of the extracellular matrix, including collagen and proteoglycans, thus promoting the structural changes necessary for tumor cells to invade surrounding tissues and spread to distant sites. Elevated levels of MMPs are often observed in dysplastic and malignant lesions and can indicate an increased risk of malignant transformation. MMPs, particularly MMP-9, can be used as biomarkers for monitoring the progression of precancerous conditions. High MMP-9 levels in tissue or saliva samples suggest active matrix remodeling and an increased likelihood of invasion and metastasis. Therefore, assessing MMP levels can help in identifying high-risk precancerous lesions and tracking disease progression. This information is valuable for early intervention and for tailoring treatment strategies to prevent the transition from a precancerous state to overt malignancy. Monitoring MMPs can also provide insights into the effectiveness of therapeutic interventions aimed at inhibiting matrix degradation and cancer spread. 20,21

Diagnostic tools and methods

Immunohistochemistry (IHC): Immunohistochemistry is used to detect the expression of specific proteins in tissue biopsies. This method utilizes antibodies that bind to target proteins, allowing for their visualization under a microscope. Through the use of chromogenic or fluorescent detection systems, IHC enables the qualitative and quantitative assessment of protein expression within tissue samples. Markers such as p16INK4a and Cyclin D1 can be evaluated using IHC, providing crucial information about the presence and level of these proteins in precancerous lesions. By assessing the expression of p16INK4a, IHC helps determine the loss of this tumor suppressor protein, which is indicative of dysplastic changes and an increased risk of malignant transformation. Similarly, the detection of elevated Cyclin D1 levels through IHC can reveal abnormal cell cycle regulation and excessive cell proliferation, both of which are hallmarks of precancerous conditions and potential malignancy. IHC aids in the diagnosis and risk assessment of malignant transformation in precancerous conditions by providing a detailed and localized view of protein expression patterns. This information is invaluable for pathologists and clinicians in making informed decisions about patient management, including the need for closer surveillance, additional diagnostic testing, or early therapeutic interventions.[22]

Polymerase Chain Reaction (PCR): Polymerase chain reaction is used to detect gene mutations, such as TP53 and CDKN2A, as well as to analyze microRNA expression. This method allows for the precise and sensitive detection of genetic

and transcriptomic changes associated with precancerous conditions. PCR amplifies specific DNA or RNA sequences, making it possible to identify even minute quantities of target genetic material in a sample. For detecting gene mutations, PCR can be used to amplify and then sequence specific regions of the TP53 or CDKN2A genes to identify alterations that may indicate an increased risk of malignant transformation. Mutations in these genes can disrupt their normal tumor-suppressive functions, leading to uncontrolled cell growth and potential cancer development. In addition to detecting mutations, PCR can be used to quantify the expression levels of microRNAs, such as miR-21 and miR-34a. By converting RNA into complementary DNA (cDNA) through reverse transcription and then amplifying the cDNA, PCR allows for the accurate measurement of microRNA levels. Changes in microRNA expression can provide insights into the molecular mechanisms driving precancerous conditions and help identify lesions with a higher likelihood of progressing to cancer. Overall, PCR is a powerful tool in the diagnosis and monitoring of precancerous conditions, offering high sensitivity and specificity in detecting critical genetic and transcriptomic alterations.[23]

Next-Generation Sequencing (NGS): Next-generation sequencing methods enable deep analysis of genomic and transcriptomic changes in precancerous lesions. NGS provides detailed information on mutations, epigenetic modifications, and gene expression changes, offering a comprehensive view of the molecular landscape of precancerous conditions. This high-throughput technology allows for the simultaneous sequencing of millions of DNA or RNA fragments, making it possible to identify a wide array of genetic alterations, including single nucleotide polymorphisms (SNPs), insertions, deletions, and copy number variations. NGS can detect mutations in key genes such as TP53 and CDKN2A, providing insights into the genetic basis of disease progression. Additionally, NGS can reveal patterns of DNA methylation and histone modifications, which are critical for understanding epigenetic regulation in precancerous lesions. By analyzing these modifications, researchers can identify epigenetic markers that may contribute to the malignant transformation of cells. Furthermore, NGS allows for the profiling of gene expression changes by sequencing RNA transcripts. This transcriptomic analysis can identify differentially expressed genes and non-coding RNAs, such as microRNAs, that play roles in the development and progression of precancerous conditions. Understanding these expression changes can help in identifying potential therapeutic targets and biomarkers for early diagnosis. The detailed information provided by NGS enables the development of personalized diagnostic and treatment strategies for precancerous conditions of the oral mucosa. By tailoring interventions based on the specific molecular characteristics of a patient's lesion, clinicians can improve the effectiveness of treatments and potentially prevent the progression to malignancy. NGS thus represents a powerful tool in precision medicine, offering new avenues for early detection, risk assessment, and targeted therapy in oral precancerous conditions.[24]

Conclusion

Molecular markers provide crucial information for the early diagnosis and management of precancerous conditions of the oral mucosa. Their integration into clinical practice can significantly improve patient prognosis by enabling more accurate and timely intervention. Further research is needed to refine and standardize the use of these markers in routine clinical practice.

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