Review Article

Molecular Biomarkers for Diagnosis of Oral Cancer: An Overview

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Abstract:

Oral cancer is one of the six leading cancers in the world and is a constant threat to the health sector in developing countries as well as developed ones. Late presentation, due to lack of awareness and invasive incisional biopsy is the crucial factor for this. Nowadays, scientists are trying to find out an easy and reliable method of early diagnosis of oral cancer and molecular biomarkers might be very helpful for that. This review was aimed to evaluate the published literature on molecular biomarkers which are related to oral cancer. For this, advanced searching was applied by specific keywords in PubMed-Medline resource database and found 12466 publications were clinical trials on humans. Then after applying all inclusion criteria, 19 articles were included finally in the review. This paper uncovered that recognition of biomarkers will be useful for the early detection of oral cancer and their prognosis after treatment. We can suggest that p53, EGFR, miR-34a, miR-143 estimation is important to decide the conceivable risk of oral malignant growth advancement in the speculated oral lesion and after the curative procedure EGFR, Podoplanin and miR-21can aid us regarding the prognosis of patient.

Keywords: Oral cancer, biomarkers, mouth ulcer, oral squamous cell carcinoma.

Introduction:

Oral cancer is a constant threat to health services in developing countries as well as developed ones. It is the 6th most common of all cancers and more than 405,000 cases are registered annually.[1] If Cancer involves the visible parts of the oral cavity such as the lip, the tip of the tongue, it can be easily diagnosed. However, when it involves the posterior one-third of the tongue, retro-molar area and the areas that can't be visible easily it delays the diagnosis. Another cause of oral cancer detection at the advanced stage is the lack of awareness of risk factors and symptoms of the oral precancerous lesion by dentists or other healthcare providers.[2] The most commonly applied diagnostic technique for detecting oral carcinoma and precancerous lesion is incisional biopsy followed by histopathology.[3] This is an invasive, painful and costly procedure so there is low patient acceptance.[4]Molecularbiomarkers are ideal for the screening of oral lesions and diagnosis of oral cancer. Early detection of oral mucosal changes and identifying biomarkers can decrease the incidence of oral cancer.[5] The aim of the article is to review the findings of the clinical trials based on the oral cancer biomarkers.

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Methods:

An advanced search was applied in the PubMed-Medline resource database. The keywords were "Oral cancer biomarkers", "Oral cancer gene". Manually selected papers are included according to eligibility criteria. MeSH (Medical Subject Headings) were applied to search the keywords: "oral" OR "mouth" AND "cancer" OR "tumor" AND "biomarkers" and they were recorded for evaluation.

Inclusion Criteria:

1. Publication related to oral cancer and molecular biomarkers.
2. Published in the previous 5 years.
3. Clinical Human Trials.
4. In English language.

Exclusion Criteria:

1. Other than clinical trials.
2. Not having access of abstract in English.
3. Published before January 2015

Results

Firstly, an electronic search was performed by keywords in PubMed-Medline and we found 12466 publications related to human trials. Out of which 367 of them were selected because they were published after January 2015. Then 234 articles were selected which met all inclusion criteria and finally 19 of those articles were selected for review.

Biomarkers are molecules that recognize the presence of a tissue. Tumor markers are tumor indicators and can be found in blood and also from ascitic fluid. They can also be found in a tumor cell or normal cell and high level of markers indicate the presence of the malignant cell. For example, p53 (also called tumor protein 53) is a tumor suppressor gene and it regulates several mechanisms including cell differentiation, DNA repair, apoptotic process etc. This p53 gene causes DNA repair and some pathogens are directly involved with this gene for inactivating it, for example, Human Papillomavirus (HPV). For this, p53 is the most widely studied biomarker of the orofacial region.

Some studies found a significant correlation between p53 and prognosis. Overexpression of the p53 gene has the direct relationship with poor prognosis in terms of survival.[6,7] Another marker is the apoptotic process inhibitor expressed in about 80% of the OSCC patients.[8] Thus, we have the chance to investigate many biomarkers to see the relation with oral cavity cancer. We conducted this review in order to see the relation of many other biomarkers with oral cancer. Table 1 shows the altered biomarkers of oral cancer in selected studies with their sample preparation and results.

Discussion:

This article reviewed the publications that where human clinical trials were conducted for the detection of biomarkers in the early diagnosis of oral cancer and their relation to them.

The p53 gene is associated with DNA repair, apoptosis and cell cycle arrest. It is a tumor suppressor gene and its inactivation correlates with increase recurrence, poor prognosis and poor survival rates in HNSCC. Gupta et al. showed that TP53 overexpression significantly related to treatment response and quality of life. When expression level increases, the response decreases. Overall survival rates were shown to be higher when expression is low.[9] In 2015, Zedan et al found that expression of p53 protein has many effects on cellular processes including carcinogenesis and differentiation.[10] Another cohort study of 77 oral leukoplakia (OL) patients in Italy showed that the expression of p53 is inversely related to clinical response. It also suggested that overexpression of p53 alone does not indicate that those OL will turn to OSCC but the combined effect of p53 and Ki-67 proteins is considered as an early diagnostic feature for identifying lesions having a risk of developing OSCC. [11]

He et al. conducted a comprehensive bioinformatics study on microarray data of 326 OSCC samples with 165 normal tissues. They identified overexpression of MMP9 in about 90% of OSCC samples. They recommend that MMP9 expression with other biomarkers like BGH3, PDIA3 is increased in the precancerous stage and further OSCC.[12]

Monteiro et al. performed a study to detect MMP9 with P63 and Podoplanin (PPN). MMP9 was found in tumor cell cytoplasm in 83.7% of cases. They also found that PPN and MMP9 both were associated with lymph node metastasis and in combination may cause tumor progression and dissemination of OSCC.[13]

Epidermal growth factor receptor (EGFR) is a transmembrane receptor protein. Solomon et al.

investigated that EGFR is the most expressed biomarker by the tumor cells. In that cohort study, EGFR was positive in 84% of cases in immunohistochemical reactions.[14] So, it will consider as a hallmark of OSCC. The authors also stated a high expression of EGFR in the pre-treatment biopsy may predict the less radiotherapeutic effect, advanced lymph node involvement and poor prognosis. According to Gupta et al. EGFR overexpression occurred in 87% of OSCC patients. So, besides p53 EGFR plays a great role as a prognostic biomarker in locally advanced OSCC patients. [9] Another research of 64 cases of oral cancer patients where tissues were immunostained with polyclonal EGFR antibody showed a significant association between EGFR overexpression and primary recurrence of OSCC. [15]

Micro RNAs (miRNAs) are single-stranded RNAs. They are non-coding and acts as a gene regulator. Various miRNAs have shown the association with oral cancer. In 2016, Patel et al. stated that miR-31 causes tumorigenesis in OSCC.[16] Cautinho-Camillo et al. observed upregulation of miR-21 in OSCC by using real-time PCR. It showed that miR-21 overexpression indicates poor survival and less therapeutic outcome.[17] miR-92b upregulation was done by another study in the same method and found tumorigenesis and cell proliferation by this biomarker.[18]

Some micro RNAs also seem to be downregulated in oral cancer. Manikandan et al. showed that miR-34a and miR-143 were significantly downregulated in cancer compared to normal tissue.[19] Another study of miRNAs done on saliva by Agilent miRNA microarray platform and found that miR-139-5p was significantly reduced in the OSCC patients than controls.[20]

Effect of miR-203 on human oral cancer cells were seen in a study conducted in Korea by Lee et al. Study showed that expression of miR-203 was significantly downregulated in OC cells compared to normal human oral keratinocytes. They also suggested the possible application in anti-cancer therapeutics.[21] In 2019 Chen et al found that Alpha-protein kinase 1 (ALPK1) expression was related to OSCC strongly for advancing of the disease process and lymph node metastasis. ALPK1 depletion showed a significant reduction in cell growth, tumor cell migration and even invasion ability. [22]

Saintigny et al. evaluated the role of MET (also called tyrosine-protein kinase) expression using immunohistochemistry in 120 patients. Multivariate analysis was used to see the association with oral cancer development. In this study, they also used gene expression profiles of 86 patients with oral leukoplakia and measured the outcome of chemoprevention trial of oral leukoplakia

Table 1: Altered biomarkers in oral cancer (biomarkers measurements and results)

Gene marker	Authors	Sample and Method	Results
p53	Gupta et al. (2015)	Immunohistochemical staining	p53 indicated overexpression in 37-75.8% oral cancer (OC)
	Zedan et al. (2015)	Immunohistochemical staining and FISH	p53 immunoreactivity in well differentiated OSCC showed highest
	Gissi et al. (2015)	HE histopathological analysis and immunohistochemical analysis	Expression of p53 inversely related to clinical response
MMP9	He et al. (2016)	Human biopsy sample, Immunohistochemical staining	MMP9 expression increased in pre-cancerous stage and further OSCC than normal tissue
	Monteiro et al. (2016)	Immunohistochemical staining	MMP-9 and Podoplanin together could contribute to tumor progression and dissemination of OSCC
EGFR	Solomon et al. (2016)	Immunohistochemical staining	Advanced lymph node involvement and poor prognosis
	Gupta et al. (2015)	Immunohistochemical staining	Poor response to chemotherapy
	Mehta et al. (2015)	Immunostained with polyclonal EGFR antibody	Recurrence of OSCC

Gene marker	Authors	Sample and Method	Results
miRNA • +miR-31	Patel et al (2016)	Human tissue, qRT-PCR	OSCC tumorigenesis
• +miR-21	Cautinho-Camillo et al.(2015)	Human biopsy, qRT-PCR	Indicates poor survival and therapeutic outcome
• +miR-92b	Liu et al.(2015)	qRT-PCR	Induces cell proliferation
• -miR-34a, -miR-143	Manikandan et al. (2015)	Human biopsy, TaqMan miRNA assays	miR-34a, miR-143 significantly downregulated in oral malignancy
• -miR-139-5p	Duz et al. (2016)	Saliva, Agilent miRNA microarray platform (V19) qRT-PCR	Expression of miR-139-5p was reduced in OSCC saliva samples
• -miR-203	Lee et al.(2015)	qRT-PCR	miR-203 expression was downregulated significantly in human oral cancer cells
• -ALPK1	Chen et al (2019)	Immunohistochemical staining, Western blot analysis	Causes reduction of the cell migration and invasion ability of OSCC
MET	Saintigny et al. (2017)	Immunohistochemical staining	MET may represe4nt as an early driver in OPML and OC risk
OPN (Osteopontin)	Luo et al. (2015)	Human OC biopsy formalin fixed and paraffin-embedded Western blot	Tumor OPN plays an important role in tumordevelopment particularly in tumor invasion and metastasis
VEGF	Kim et al. (2015)	Immunohistochemical staining qRT-PCR	Tumor angiogenesis and progression of OSCC
Podoplanin (PPN)	Grochau et al. (2018)	Immunohistochemical staining	Important role in malignant degeneration and advancement
	Pradhan et al. (2018)	Immunohistochemical staining	Expression of PPN associated with the degree of differentiation of OSCC

+ =Upregulation, - =Downregulation

and normal mucosa. Results showed that those with the increased risk of oral cancer had 163 transcripts overexpressed compared to normal mucosa where they identified 23 overlapping transcripts, including MET. This data suggested that MET activation may be an early driver of oral pre-malignancy and a target for chemoprevention of oral carcinoma.[23]

Luo et al. investigated the role of Osteopontin (OPN) in the chemo-sensitivity of oral squamous cell carcinoma (OSCC). The authors took 121 patients and attempted to find out the role of OPN in cell proliferation. Recombinant human OPN cells were taken from the human tongue carcinoma cell line and investigated whether there is an influence of OPN protein in the proliferation of these cells. In the study, an increased percentage was found in the carcinoma cell which indicates the major role of OPN to promote the growth of OSCC cells.[24]

Kim et al. investigated the expression of vascular endothelial growth factor (VEGF) in OSCC cells by immunohistochemical staining and qRT-PCR. They found a significant correlation between VEGF expression and histologic differentiation of tumor size. VEGF also plays a role in tumor angiogenesis and the progression of oral cancer.[25]

Podoplanin is a transmembrane sialoglycoprotein and its expression is also considered as a potential biomarker in the assessment of the risk of OSCC. Grochau et al. examined the patients of oral leucoplakia in a prospective study. A significant association was observed between PPN expression and degrees of dysplasia. It seems to play an important role in malignant degeneration and advancement.[26] Recently Pradhan et al. showed that PPN expression was seen in 75% of cases and it was associated with the degree of differentiation of the tumors. Also, expression of SOX2 was found in 63% of OC cases.[27] Analysis of PPN thus can be an aid to prognosis and treatment of OSCC.

These studies revealed that alteration of genomic proteins (p53, MMP9, EGFR, micro RNAs, MET, Osteopontin, VEGF, Podoplanin) have a correlation to tissue transformation in creating oral cancer. Proper knowledge of this protein expression may lead to the possibility of oral cancer prevention and determining the prognosis of treatment. In this review statistical analysis was not possible because of a lack of homogeneity among the studies. They have a different sample sizes, methodologies and techniques of identifying gene expression. All of these studies were done from human tissue samples except one where saliva was examined for biomarker.

Conclusion

Early detection of any premalignant lesion and oral cancer is a very crucial part of patient survival. Molecular biomarkers have great potential in screening oral cancer and good prognosis after treatment. From this review, we can recommend that p53, EGFR, and micro RNAs such as miR-34a, miR-143 measurement is necessary to determine the possible risk of oral cancer development in the suspected oral lesion and after the curative surgery EGFR, Podoplanin and miR-21can tell us about the prognosis of the patient. Further study in this field is essential considering the larger sample size and to find out the underlying mechanism of biomarkers.

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