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REVIEW ARTICLE

Recent progress of biomarkers in oral cancers

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Abstract: Oral cancers are the seventh most common cancer globally. While progresses in oral cancer treatment have been made, not all patients respond to these therapies in the same way. To overcome this difficulty, numerous studies have been devoted to identifying biomarkers, which enable early identification of patients who may benefit from a particular treatment modality or at risk for poor prognosis. Biomarkers are protein molecules, gene expression, DNA variants, or metabolites that are derived from tumors, adjacent normal tissue or bodily fluids, which can be acquired before treatment and during follow-up, thus extending their use to the evaluation of cancer progression and prediction of treatment outcome. In this review, we employed a basic significance level (<0.05) as the minimal requirement for candidate biomarkers. Effect sizes of the biomarkers in terms of odds ratio, hazard ratio, and area under the receiver operating characteristic curves were subsequently used to evaluate the potential of their clinical use. We identified the CCND1 from the tumor, human papillomavirus, HSP70, and IL-17 from the peripheral blood, and high density of CD45RO$^+$ tumor-infiltrating lymphocytes as the clinically relevant biomarkers for oral cancers.

Keywords: Biomarkers; Cytokines; Human papillomavirus; Oral cancers; Pharmacogenomics

1. INTRODUCTION

Oral cancer remains a deadly malignancy worldwide, accounting for more than 52 000 people dies from this type of cancer and 28.6% of all deaths in 2019 in Taiwan.[1,2] Oral cancer mainly occurs in the mucosal surfaces of the oral cavity, pharynx (throat), larynx, paranasal sinuses, nasal cavity, and salivary glands. A majority of oral cancers originate from the squamous cells (>90%).[3] The incidence of developing oral cancers is significantly higher in males than females, with a ratio ranging from 2:1 to 4:1.[4] Consumptions of betel quid and smoking are the major risk factors of oral cancer in Taiwan and Southeast Asia,[5,6] while alcohol and tobacco consumptions are risk factors in western countries. Additionally, viral infections of the human papillomavirus (HPV) and Epstein-Barr virus are also important risk factors for the development of oral cancers. Particularly, HPV accounts for a large percentage of oral cancers in western countries. Food-related carcinogenesis of oral cancers has gradually gained public awareness, while the carcinogenesis by invisible viral infections received less attention and became more problematic.[1]

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Biomarkers are important components of precision medicine, as they provide indicators and guides to ensure prevention, early detection, and personally optimized treatments of the disease. Biomarkers are often obtained from body fluids or solid tissues, where the measured values either indicate the quantified risk of diseases and/or the outcome of treatments. Here we review the current status of biomarkers in oral cancers. Literature was searched by PubMed in May 2021. We use $p < 0.05$ in the univariate analysis as the minimal requirement of evidence for candidate biomarkers to be reviewed. These candidate biomarkers were further evaluated in terms of their effect sizes, such as odds ratio, hazard ratio, and area under the receiver operating characteristic curves (AUC).

2. BIOMARKERS IN THE PERIPHERAL BLOOD

The peripheral blood is an important source of biomarkers, including host and viral DNA, RNA, proteins, cells, and metabolites. HPV is a DNA virus from the *Papillomaviridae* family. The viral genome of HPV can be assayed effectively in peripheral blood using polymerase chain reactions.[7,8] The detection of HPV not only reveals the etiology of oral cancers but also reflects the subsequent prognosis. The p16 protein (*a.k.a.* CDKN2A) expression in the tissue is a highly related surrogate biomarker for HPV,[9] with 80% to 90% concordance between the HPV and p16 detections.[10] The p16 levels were associated with better treatment outcomes.[9,11–17] The function-disrupting somatic mutations of CDKN2A, on the other hand, are associated with poor prognosis.[18]

Apart from the viral genome, the human genomic DNA is commonly extracted from peripheral blood mononuclear cells (PBMC) for investigations. The human genomic DNA encodes the blueprints of the body in health and disease, and germline variants reflect personal variabilities. Hence, genomic DNA has been interrogated for dinging indicators of the occurrence

of oral cancers. Variants of genes in the DNA repair pathways, comprising (1) base excision repairs; (2) nucleotide excision repairs; and (3) double-strand break repairs, have been ascribed to oral cancers.[19–21] A meta-analysis of case-control studies up to 2010 showed that exonic variants of XRCC1 codon 194 and 399, and the Asp312Asn variant of XPD, are repetitively associated with oral cancer occurrence.[20]

Human genomic DNA obtained from the peripheral blood has also been investigated for finding biomarkers pertaining to patients' prognosis given specific treatments (Fig. 1). Germline variants in genes related to the cell cycles, apoptosis, and the maintenance of cellular integrity, such as Tp53, ATM, BCL2, TGFβ, were shown to correlate with treatment outcomes.[22] Variants of *EGFR*, *kRas*, and *FCGR2A* genes were associated with skin toxicity by the treatment of cetuximab, an EGFR antagonist.[23]

Serum proteins represent one major category of biomarkers. Soluble heat shock protein 70 (HSP70) concentrations, quantified by enzyme-linked immunosorbent assay, were elevated in NHC patients but not in healthy controls (patient number: 21 vs 28, AUC = 0.91, $p < 0.0001$).[24] It was also shown to correlate with tumor levels before treatment.[24] The serum level drops after the surgery and radiotherapy, while the anti-HSP70 autoantibodies remained stable.[24] Hence, soluble HSP70 may serve as a biomarker for early diagnosis of oral cancer occurrence.[24]

Biomarkers reflecting the immunological and inflammatory status, such as cytokines and their receptors, are important for oral cancer diagnosis and prognosis.[25] Cytokines are soluble proteins with low molecular weights. The soluble form of interleukin-2 receptor was shown to be higher in oral cancer patients than in healthy controls and higher in stage 3 and 4 oral cancer patients than in stage 1 and 2 patients.[3] Serum IL-17 concentrations have been shown to be an effective clinical biomarker for indicating imminent hepatocellular carcinoma.[26] A recent study investigated oral cancer biomarkers pertaining to the inflammation-related T cell biology in a total of 120 oral cancer patients and 24 healthy controls in Taiwan. Among them, 72 oral cancer patients were divided into two groups based on their number of Th-17 cells, CD8+IL-17 cells, and all IL-17+ cells of the peripheral blood. All these patient strata manifest distinct overall survival after surgery.[27] Particularly, patients with more IL-17-expressing cells among PBMCs have a significantly poorer 5-year overall survival (hazard ratio = 2.591, 95% confidence interval: 1.27–5.28, $p = 0.009$). Furthermore, the percentages of IL-17+ cells among PBMC, CD4+IL-17+ cells among all CD4+cells, and CD8+IL-17+ cells among all CD8+cells are all significantly lower in healthy controls and higher in advanced oral cancers (Table 1).[27]

3. BIOMARKERS IN CANCER TISSUES

Tissues are full of molecular characteristics directly involved in all hallmarks of cancer, such as uncontrolled cell cycle, invasiveness, metastasis, and anti-apoptosis. Somatic mutations occur in the DNA of the malignantly transformed cells and tissues. A study employing targeted next-generation sequencing of a panel of 100 cancer-related genes revealed that a higher tumor mutation burden was observed in HPV-negative patients.[18] Many somatic, function-disrupting mutations of oral cancers occur in genes that protect the integrity of the cell, such as Tp53, and the mutations were correlated with poor prognosis.[18] The lower protein level of Tp53 was also associated with a poorer prognosis.[28] On the other hand, CCND1, a cell cycle controlling gene, is frequently observed with focal amplification, which is associated with poor survival.[18] The protein level of CCND1 by immunohistochemistry (IHC) staining is also associated with lymph node metastasis and poor overall survival,[29–31] EGFR mutations,[41] DNA copy number changes,[17,42] and protein level[9,43,44] are responsible for poor prognosis. ERCC1 proteins[35–39] and RNA expressions[40] in tissues were associated with unfavorable outcomes. Somatic amplification of FGFR1 and protein levels were found to be associated with poor prognosis.[18,46]

Immunological protein expressions in the tumor level may be associated with a good prognosis. IL-24 levels measured by IHC were shown to correlate with better outcomes and reduced incidence of second primary malignancies in the oral region.[32] IL-24 is mainly expressed in the cytosol of the cell.[32] Levels of immunological checkpoint proteins may correlate with poor prognosis. High PD-L1and PD-L2 levels in tumor specimens were correlated with poor overall survival.[33,34] The level of PD-L1 in the primary site was lower than in the metastasis site.[34] CD44 protein level is associated with poor survival.[9] The chemokine CXCL12 (*a.k.a.* SDF-1) and its receptor CXCR4 was reported to be associated with local-regional control of patients treated with resection and adjuvant radiotherapy.[47,48] PITX2 hypermethylation was associated with better overall survival.[49] MRP2 level positivity correlates with the good outcome by concomitant cisplatin-based chemoradiation.[50] The abundance of antioxidant GST protein is positively associated with resistance to therapeutic agents.[51] ERCC1, XIAP, CIAP, and XPA protein overexpression is associated with poor prognosis.[39,52,53] The high abundance of PTEN was associated with poor prognosis in patients with cetuximab-based chemotherapy.[54] The LYPD3 gene encodes the C4.4A protein, a highly glycosylated glycosylphosphatidylinositol-anchored protein homologous to the urokinase receptor, is reported to be associated positively with poor overall survival post-surgery.[55] The KLK6 protein can prevent epithelial-to-mesenchymal transition, and its protein level in the primary tumor was associated with a good prognosis.[56] Cholinesterases, including acetylcholinesterase and butyrylcholinesterase, were shown to have reduced activities in tumor tissues.[57] A review of endogenous markers of hypoxia measured using IHC showed that the expression of HIF-1a, HIF-2a, CA-IX, GLUT-1, and OPN might indicate poor outcome, particularly in patients treated by surgery only.[58] Note the hazard ratios in this review was defined to non-expressions.[58]

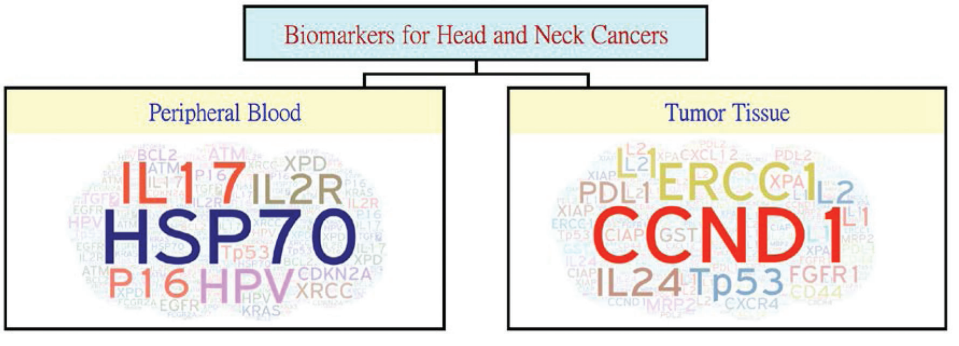

Fig. 1 The candidate biomarkers in the peripheral blood and the tumor tissues of oral cancer patients.

Table 1

Candidate biomarkers in peripheral bloods and tumor tissues with their effect sizes

Oral cancer Biomarkers	Indications	Max effect size reported	Type	Ref
Peripheral blood				
HSP70	Occurrence	AUC: 0.91	Protein	24
XPD	Occurrence	OR: 1.14	Germline variant	20
XRCC	Occurrence	OR: 1.50	Germline variant	20
ATM	Occurrence	OR: 4.43	Germline variant	22
HPV	Occurrence/prognosis	HR: 0.34	DNA	7,8
IL-17	Prognosis	HR: 2.591	Cell	27
IL-2R	Prognosis	NA	Protein	3
BCL2	Prognosis	HR: 0.32	Germline variant	22
TGFβ	Prognosis	HR: 0.21	Germline variant	22
EGFR	Treatment side effect	OR: 0.35	Germline variant	23
kRas	Treatment side effect	OR: 0.27	Germline variant	23
Tumor tissues				
CCND1	Prognosis	HR: 3.06	Protein	28–31
FGFR1	Prognosis	HR: 3	Somatic amplification	18
IL-24	Prognosis	NA	Protein	32
PD-L1	Prognosis	HR: 2.06 (for smokers)	Protein	33,34
PD-L2	Prognosis	NA	Protein	34
p16 (CDKN2A)	Prognosis	HR: 0.34	Protein	–
Tp53	Prognosis	HR: 1.95	Somatic mutation/protein	18,28
ERCC1	Prognosis	HR:3.0	Protein/mRNA	35–40
EGFR	Prognosis	HR: 2.943	Somatic mutation/protein	9,17,41–44
Saliva				
Lactic acid	Occurrence	AUC: 0.80	Metabolite	45
Valine	Occurrence	AUC: 0.81	Metabolite	45

AUC = area under the receiver operating characteristic curve; HR: hazard ratio; OR = odds ratio.

4. BIOMARKERS IN TUMOR MICROENVIRONMENT

The tumor microenvironment (TME) is an ensemble of non-cancer cells, predominantly the stromal and immune cells, which intimately interact with tumor cells to influence the outcome of cancer progression. The cellular and extracellular components in TME are of clinical significance as biomarkers for predicting the treatment efficacy and outcome. Most biomarkers of TME relate to overall immune status, of which the pattern of tumor immune infiltration has been closely associated with clinical treatment response. Indeed, not only a high density of tumor-infiltrating lymphocytes (TILs) but also the phenotype of immune infiltrate is crucial for superior prognosis of oral cancers.[59–61] Specifically, a higher amount of CD3 or CD8 T cell and CD57 natural killer (NK) cell infiltrates has been linked to better overall survival and progression-free survival of oral cancer patients.[62] On the contrary, a predominance of tumor infiltrates rich in Treg or $CD4^+$ Th2 lymphocytes inversely correlated with patient outcome. Recently, the immune status of TME based on the density of $CD8^+$ and memory $CD45RO^+$ T cells in the center and invasive front of tumor has been used to estimate the Immunoscore as a biomarker for prediction of treatment response.[63] Zhou et al evaluated Immunoscore in 169 patients with oral cancers and found that a high density of $CD45RO^+$ TILs within cancers was significantly associated with recurrence-free survival ($p = 0.0018$ and 0.0007 by log-rank test),[64] and the results of which were consistent with other studies.[65–68]

In addition to surface biomarkers of immune cells, the immune gene expression of TME as therapeutic or prognostic biomarkers has also been assessed. Yao et al[69] used high-throughput RNA sequencing data and identified four immune-related genes (*PVR*, *TNFRSF12A*, *IL21R*, and *SOCS1*) that were significantly correlated with overall survival. Huo et al. also used the gene expression data from 816 oral cancer patients to establish a prognostic risk model based on TME gene signature, known as the TMEscore, showing that gene signature of TME is of potential to be prognostic biomarkers.[70] Effective tumor-associated immune response often involves the clonal expansion of specific antigen-reactive T cells, and therefore, the diversity of immune repertoire in TME is supposed to be a type of biomarkers for predicting the efficacy of immunotherapy. Interestingly, while some studies demonstrated a positive correlation between T cell clonality and treatment response, some studies found that intratumoral T cell clonality is less correlated with patient survival.[71–74] To date, whether the richness and clonality of T cells within TME predict the treatment response of oral cancers remain unclear, with only a few studies investigating the correlation between T cell receptor (TCR) richness in peripheral blood with therapeutic response. In this regard, a study from Kansy et al[75] found that head and cancer patients who exhibit an increase in the number of unique TCR sequences in peripheral blood after cetuximab-based neoadjuvant treatment have a better prognosis than those without significant change in T cell repertoire, thus concluding that TCR diversity could represent as a novel biomarker for monitoring the response of patients to cetuximab-based neoadjuvant treatment.

5. BIOMARKERS FROM OTHER SOURCES

Saliva represent one additional, easily accessible source of biomarkers. Studies reveal that metabolites such as lactic acid and valine can indicate occurrence of oral cancers.[45] Additionally, lycine, proline, citrulline and ornithine were found to be associated with early stage oral cavity squamous cell carcinoma.[76]

6. DISCUSSIONS

Peripheral blood, TME, and tumor tissues are the major sources of cancer biomarkers. The blood and TME former contain

many immunological signals to interact with tumor cells. The tumor tissue carries various unique or differentially expressed molecules that are relevant to treatment response and patient prognosis. In this study review, we identified HPV, HSP70, and IL-17 from the peripheral blood, CCND1 from the tumors, and the density of $CD45RO^+$ TILs as the clinically relevant biomarkers for oral cancers. Apart from blood and tissues, biomarkers can also be identified in saliva or from microbiota. However, the studies were relatively scarce, and solid evidence to support the role of saliva or microbiota as a biomarker is lacking. On the other hand, due to the complexity of TME and the heterogeneity of tumor cells, a single biomarker may not be able to effectively monitor cancer development, progress, or treatment response. Considering the fact that distinct markers have different sensitivity to cancer progression and outcome prediction, combining multiple markers seems to be a reasonable strategy. Meanwhile, cancer biomarkers should be selected according to the stages or types of cancers and the intention for determining treatment modalities, cancer follow-up, or prognosis prediction.

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