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Updates of liquid biopsy in oral cancer and multiomics analysis

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Abstract

Liquid biopsy is a method sampled from body fluids, such as blood, saliva, urine, pleural effusion, cerebrospinal fluid, and so on. It is minimally invasive and reproducible and therefore can build a dynamic, real-time monitoring of oral squamous cell carcinoma patient's conditions and treatment responses. Circulating tumor cells, circulating tumor DNA and exosomes are three main detection objects of liquid biopsy, having different detection methods and features involving cost, sensitivity, specificity and output. Blood and saliva are the options of liquid biopsy in oral cancer. Then we reviewed the studies of liquid biopsy in oral cancer, integrating multiomics analysis of these results. The multiomics analysis of genomics, transcriptomics, proteomics, metabolomics, and DNA methylation have shown potential for the early screening, diagnosis, staging, prognosis, personalized medicine therapy, and monitoring of recurrence (minimal residual disease). Besides, we concluded some problems to be solved, such as the lack of the standard of the measurement methods and procedures of samples, the insufficient connection among different omics, and how to improve the sensitivity and specificity. And we also put up rough assumptions to these problems. However, the analysis of multiomics of liquid biopsy in oral cancer still shows great clinical value in the diagnosis and treatment of oral squamous cell carcinoma.

KEYWORDS
liquid biopsy, minimal residual disease, multiomics, oral squamous cell carcinoma, precision medicine

1 | INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of oral and maxillofacial regions. In recent years, the survival rate of OSCC patients hasn't shown a satisfying promotion in the consideration of the rapid development of precision medicine. There are still many issues that remained to be resolved. First of all, due to the lack of an effective and repeatable way to detect OSCC in real time, we don't have enough understanding of the spatial and temporal heterogeneity of OSCC. It will lead to difficulties in the diagnosis and therapy of OSCC. Secondly, biopsy sometimes is hard to get enough specimen through surgery. The minimally invasive method needs to be applied. Another challenge in OSCC is how to identify OSCC in early stages. Because of the delayed detection, some patients may miss the optimal time for treatment, which results in the unsatisfying survival rate of OSCC. And as so far, we can't real-time monitor treatment responses and resistance of OSCC by repeated analysis. We also need a shorter turnaround time for genotyping mutations. And liquid biopsy may solve part of above issues.

2 | LIQUID BIOPSY

Liquid biopsy is a method whose samples come from body fluids, such as blood, saliva, urine, pleural effusion, cerebrospinal fluid, and so on (Siravegna et al., 2017). According to the contents of body fluids, we can acquire information to assist in the early screening, diagnosis, staging, prognosis, personalized medicine therapy, and monitoring of recurrence of tumors (Soda et al., 2019). Because the sampling of liquid biopsy is minimally invasive, or even non-invasive,

there are a few apparent advantages compared to traditional tissue biopsy. First of all, liquid biopsy is adaptable for more patients due to less damage for patients during the sampling. Besides, liquid biopsy can be used in a patient repeatedly, which is impossible in tissue biopsy. The samples of patients at different time can help us build a dynamic, real-time monitoring of patient's conditions. It is of great significance for the selection of therapy. It is also convenient and more acceptable for patients. And no matter where tumors are, we can still get body fluids samples. In liquid biopsy, circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and exosomes are three most common biomarkers.

2.1 | Circulating tumor cells

CTCs are tumor cells released into the circulatory system spontaneously or during medical operations. They contain most of the features of primary tumors or metastases. The existence of CTCs is a prerequisite for the formation of metastases. CTCs appear not only in patients in advanced stages but also in those in early stages. If the process of CTCs coming into the bloodstream is spontaneous, CTCs need to experience the steps of the epithelial–mesenchymal transition (EMT), intravasation, and survival in blood. Before metastase, CTCs need to undergo the processes of extravasation and mesenchymal-epithelial transformation (MET) (Massague & Obenauf, 2016). Therefore, CTCs with features similar to epithelial cells which have more potential of metastase. What we need to know about CTCs is that the CTCs content of blood is extremely low. In a metastatic patient, 1 to 100 CTCs could be found per milliliter of blood, while the content of white blood cells is about 10^7 (Chen et al., 2014). The low content of CTCs requires high precision detection. How to enrich and detect CTCs accurately from blood is crucial. The involved method can be divided into antigen-dependent and antigen-independent, such as the techniques based on the density or size of cells. CTCs show great value in research because they contain lots of information about the tumor. In patients with metastatic breast cancer, Cristofanilli et al. evaluated the number of CTCs, and the results suggested that it was higher in the patients with shorter median progression-free survival and shorter overall survival (Cristofanilli et al., 2004).

To isolate CTCs, label-dependent and label-independent methods have been established. Label-dependent methods use some specific markers in CTCs to isolate them, such as EpCAM-antibody or immunomagnetic enrichment. As for label-independent methods, the principle is based on physical features, such as the size or the density (Kong & Birkeland, 2021).

2.2 | Circulating tumor DNA

Circulating tumor DNA (ctDNA), DNA fragments came from tumor cells, is one of circulating cell-free DNA (cfDNA), which is DNA fragments released from apoptotic or necrotic cells in the circulatory system (Gorgannezhad et al., 2018). ctDNA can be differential from other cfDNA by gene mutations (Lousada-Fernandez et al., 2018). Compared with CTCs, the level of cfDNA is comparative higher. The low abundance of cfDNA is approximately 30 ng in per milliliter plasma in healthy individuals and 180 ng in oncological patients. The content of ctDNA accounts for 0.01% ~ more than 10% of cfDNA (Mathai et al., 2019). The higher the tumor load patients have, the more ctDNA can be detected. So the detection of ctDNA is more accurate in advanced stages. And with the invention of ctDNA detection techniques, the precision of the detection is promoted. For example, BEAMing is a technique that absorbs ctDNA through magnetic beads. It can distinguish one sequence of ctDNA from ten thousand sequences of DNA from healthy cells. ctDNA was detectable in more than 75% of patients with advanced pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers (Bettegowda et al., 2014).

Most detection methods of ctDNA are based on PCR, such as qPCR, digital PCR and so on. And NGS is also the important method to analyze ctDNA. We can also clarify these methods into the targeted and the untargeted. Targeted methods include those based on PCR and some based on NGS. Untargeted methods include others based on NGS, such as whole genome sequencing (WGS) and whole exome sequencing (WES) (Kong & Birkeland, 2021).

2.3 | Exosomes

Exosomes refer to lipid bimolecular membrane structure vesicles with a diameter of 40–100 nm that are actively secreted by different kinds of cells. Their contents, such as proteins and RNAs, also contain the information of OSCC (Simons & Raposo, 2009). The advantage of exosomes compared to CTCs and cfDNA is that they exist more widely in body. If the detection of exosomes can be applied in clinic, it can be a non-invasive method, which means more social acceptability. It will aid in the early screening. Another advantage of exosomes is that the content is much higher than CTCs and cfDNA. In per milliliter of blood, there are more than 10^9 exosomes (Colombo et al., 2014). And considering the structure of exosomes, the lipid bilayer membrane avoids them to be disassembled too quickly in blood. The stability of exosomes protects their contents. In recent years, the researches on the detection of microRNAs (miRNAs) in exosomes show the potential in liquid biopsy. Bernard et al. used blood samples from patients with pancreatic cancer to detect DNA in exosomes. They found that the level of KRAS mutant allele frequency (MAF) from exosome DNA (exoDNA) increased in patientes after neoadjuvant therapy which meant disease progression. They also demonstrated that patients with the level of MAF from exoDNA equal or more than 5% had shorter progression-free survival and overall survival (Bernard et al., 2019).

We can isolate exosomes according to their physical features or immune affinity. Ultracentrifugation and precipitation are useful methods to isolate exosomes. The specific surface proteins, such as CD63 and CD9, also can be used to enrich exosomes. At the same time, we can detect exosomes depend on the characters of various

biomarkers in exosomes. Western blotting can be used to detect the expression of surface markers of exosomes. Mass spectrometry (MS) and liquid chromatogram-mass spectrometry (LC-MS) can detect protein in exosomes. The methods based on PCR we mentioned in the second paragraph of 1.2 can also detect nucleic acids (Kong & Birkeland, 2021).

Table 1 lists the advantages and disadvantages of various methods in liquid biopsy. Generally, label-independent CTCs detection is comparatively cheap, but it is poor in specificity. Labeled CTCs can make up for the above deficiency with high sensitivity, but the cost is relatively high. ctDNA is widely used as an important biomarker with two aspects of targeted and untargeted. There are also two different ways to detect exosomes in liquid biopsy. Between them, physical cost less with lower specificity, and immune affinity has perfect sensitivity with low output and high price. Taken together, no method is faultless. Thus, we choose appropriate test methods to satisfy our research goals (Table 1).

3 | LIQUID BIOPSY IN OSCC

3.1 | Oral squamous cell carcinoma

In OSCC, liquid biopsy is expected to be used in different stages. More and more researchers demonstrated that the results in OSCC were similar to other tumors, such as breast cancer, non-small cell lung cancer, colorectal cancer, and prostate cancer, which have already applied liquid biopsy in clinic. Liquid biopsy in OSCC also has the potential in the early screening, diagnosis, staging, prognosis, personalized medicine therapy, and monitoring of recurrence of tumors. The study showed that the number of CTCs was related to the prognosis and recurrence of OSCC patients. Liquid biopsy in OSCC can reveal the possibility of metastasis and recurrence in multiomics (Table 2).

3.2 | Early screening

One of the reasons that result in the low five-year survival rates of OSCC is the delay of diagnosis. Liquid biopsy has shown the potential to distinguish OSCC from oral leukoplakia, oral sub-mucous fibrosis, and healthy control. It can be helpful for OSCC patients to be diagnosed in early stages. At this period, some OSCC patients may not have specific symptoms and therefore may ignore or refuse to do tissue biopsy. Liquid biopsy can solve this problem because of its convenience and minimal invasion. It can identify OSCC in early stages. Bortolini Silveira et al. evaluated CTCs and ctDNA in 198 HER2-negative metastatic breast cancer patients. The results showed that only 26 patients couldn't be detected CTCs or ctDNA before therapy (Bortolini Silveira et al., 2021). A study on 10 healthy controls and 74 non-small cell lung cancer (NSCLC) patients showed that CTCs could be found in 60% of patients, while they could not be detected in healthy controls (Fina et al., 2021). In OSCC, Gai et al. found miR-302b-3p and miR-517b-3p could distinguish patients from healthy controls because they were up-regulated in OSCC patients (Gai et al., 2018).

3.3 | Monitoring and management

Liquid biopsy can monitor tumors in real time and repeatedly. It can help clinical doctors get more information about patients in their whole course of diseases, and get to know the changes of patients in time. It is useful to determine the specific plan of treatment. The response to treatment can be measured by some biomarkers, and the patients can get personalized treatment. Qvick et al. detected ctDNA of lung cancer patients in different stages. They found that the number of variants was higher in patients in stage IIIb-IV than stage I-IIIa. And the overall survival was also significantly lower when patients with more variants. It suggested that lung cancer patients with more mutational load needed closer follow-up (Qvick et al., 2021). The study of 9 metastatic prostate cancer patients showed that the dynamic pattern of some CpG sites could reflect changes of clinical events. It implied the potential of liquid biopsy in the monitoring of prostate cancer (Silva et al., 2021). Luo et al. found that the patients with higher concentrations were more likely to have a higher recurrence risk (Luo et al., 2020).

3.4 | Minimal residual disease

Minimal residual disease (MRD) may trigger metastasis and recurrence. After the treatment, there may remain a few cancer cells, which cannot be identified by some traditional detection method.

TABLE 1 Summary of different methods involving CTCs, ctDNA and exosomes in lipid biopsy

Biomarkers	Detection methods	Advantages	Disadvantages
CTCs	Label-dependent	Sensitive, high purity	Costly, low output
	Label-independent	Simple procedure, low cost	Low specificity
ctDNA	Targeted	Low cost, high sensitivity	Need prior knowledge Limited applicable scope
	Untargeted	No need prior knowledge	Less cost-effective
Exosomes	Physical	Simple procedure, low cost	Long test cycle, low specificity
	Immune affinity	High sensitivity	Low throughput, low output, costly

TABLE 2 Summary of liquid biopsy of OSCC about metastasis and recurrence within 5-year literature

Study cohort	Detection technique/ platform	Molecular indicators	Omics	Sensitivity/Specificity	AUC	Associations	Ref.
121 OSCC 50 HC	Quantitative spectrometry	cfDNA	Genomics	-	0.65	Cervical lymph node metastasis	Lin et al. (2018)
61 OSCC	qPCR-HRMA	mutant mitochondrial DNAs	Genomics	-	-	Recurrence and Distant metastasis	Uzawa et al. (2012)
114 OSCC 66 OLK 70 HC	qRT-PCR	miR–222-3p, miR–423-5p and miR–150-5p	Transcriptomics	-	-	Lymph node metastasis	Chang et al. (2018)
20 OSCC 18 HC	qRT-PCR	miR–486-5p, miR–375 and miR–92b–3p	Transcriptomics	-	-	Recurrence	Yan et al. (2017)
20 OSCC 10 HC	Western blotting	PF4V1 expression	Proteomics	65.00%/100.00%	0.808	Lymph node metastasis	Li, Zhou, et al. (2019)
159 OSCC 44 HC	ELISA	lipopolysaccharide-binding protein	Proteomics	-	-	Lymph node metastasis	Wang, Li, et al. (2020)

Abbreviations: AUC, area under the curve; HC, healthy control; OLK, oral leukoplakia; OSCC, oral squamous carcinoma.

Liquid biopsy can detect biomarkers to find out MRD. It can suggest that the patients need further treatment to kill the cancer cells. This may decrease the rate of metastasis and recurrence. Tie et al. collected plasma samples from post-operative colon cancer patients to detect MRD. In 178 patients without adjuvant chemotherapy, 79% (11 of 14) of patients who could be detected ctDNA after surgery recurred, while only 9.8% (16 of 164) of patients recurred if they were ctDNA negative after surgery (Tie et al., 2016). In Dudley et al. study, they detected urine tumor DNA (utDNA) to monitor bladder cancer. They found that 92% of patients were utDNA positive before clinical recurrence (Dudley et al., 2019). Although there was no study about MRD in OSCC, we can expect its application in OSCC.

3.5 | Saliva

An interesting thing we need to notice is that although the blood samples are the most widely used, saliva for OSCC patients may be an alternative choice considering the connection between salivary contents and OSCC. Oral tumors' locations determine that the use of saliva samples in oral cancer is a potentially practical way. Compared with blood, sampling in saliva is non-invasive and more convenient. Although the studies have been mostly on blood samples so far, some studies have been done in saliva on diagnosis, early screening, and staging of OSCC (Table 3).

The detection of saliva began in the last century or even earlier. Saliva is convenient for collection and storage. Saliva detection can be used in salivary gland diseases, some endocrine diseases, autoimmune diseases, and some other system diseases, such as cerebral palsy, Alzheimer's disease, cystic fibrosis, anorexia. Besides, it can monitor the concentration of drugs. Saliva detection also can be used in forensic. In recent years, the relationship between tumors and saliva has been valued (Lee et al., 2020). The concentrations of some nucleic acids, proteins, and metabolites are related to the conditions of tumors. However, saliva detection has been faced some challenges. One of the problems is that there are so many compositions in saliva that sometimes it is difficult to isolate the target biomarker. The compositions in saliva are variable and personalized. Thus the standard of the normal still has not been settled. In liquid biopsy, another problem of saliva detection is the consistency of the biomarkers between saliva and blood. In the future, we need to do more clinical trials of saliva detection to settle the normal range of compositions in saliva and compare the concentration of biomarkers in saliva with blood.

4 | MULTIOMICS OF LIQUID BIOPSY IN ORAL CANCER

4.1 | Genomics

The analysis of genomics can assist in the prediction of metastasis and the prognosis of OSCC. Lin et al. showed that the cfDNA plasma concentration was significantly higher in the patients with

OSCC (53.1 ± 6.69 ng/ml) than the control group (24.0 ± 3.33 ng/ml), and the area under the curve (AUC) was 0.69 when using 20.2 ng/ml as the cutoff value. cfDNA level was also higher in the patients with larger tumor size, or cervical lymph node metastasis, or in advanced stage, and the AUC of lymph node metastasis status was 0.65 when the cutoff was 42.0 ng/ml. The decrease of cfDNA level could be observed after the surgery in 75% of the patients (45 of 60). They also confirmed that the higher cfDNA plasma concentration was associated with poor prognosis (Lin et al., 2018).

Besides cfDNA, other types of DNA can also reveal the information of patients with OSCC. According to Uzawa et al. study, tumor-derived mutant mitochondrial DNAs (mut-mtDNAs) was regarded as an indicator for the prognosis. The mut-mtDNAs level was higher in the patients with poor prognosis, and the AUC values suggested it was more sensitive for the risk of recurrence or metastasis than serum SCC antigen (Uzawa et al., 2012).

4.2 | Transcriptomics

In recent years, studies about liquid biopsy in oral cancer showed the potential of transcriptomics in diagnosis and staging. In Gai et al. study, the samples from 16 OSCC patients and 6 healthy controls showed that patients and controls could be distinguished by the exist of specific miRNAs, such as miR-302b-3p and miR-517b-3p in the extracellular vesicles (EVs) of OSCC patients. They also discovered the significant up-regulation of miR-512-3p and miR-412-3p in EVs of patients by qRT-PCR, and their ROC curves showed AUC values of 0.847 and 0.871 respectively, which meant that the detection of miRNA of EVs may be a good indicator to identify patients with OSCC (Gai et al., 2018). He et al. discovered that compared to 10 healthy controls, miR-24-3p was up-regulated in salivary exosomes of 45 pre-operation patients with OSCC. After eliminating the interference of exogenous factors, the salivary exosomal miR-24-3p concentration of patients (10.13 ± 1.961) was more than five times like that in healthy controls (1.769 ± 0.5719). To evaluate the discrimination power of miR-24-3p for patients with OSCC, ROC curve was constructed with AUC values of 0.738; the sensitivity and the specificity of miR-24-3p were 64.4% and 80% (He et al., 2020). The down-regulation of some other miRNAs also could be observed in OSCC patients. Kulkarni et al. studied the concentration of exosome miR-30a sampling from the serum of patients with OSCC and healthy controls. The significant down-regulation was shown according to the samples (Kulkarni et al., 2020). In the study of 20 patients with oral sub-mucous fibrosis (OSMF), 20 OSCC patients, and 40 healthy volunteers, the miR-21 level in the serum was measured by RT-PCR. The results showed that miR-21 expression level was much higher in OSCC patients compared to patients with OSMF. The up-regulation of miR-21 level in the serum had a close connection with the clinical stages of the OSCC patients. That is to say, the more advanced stages the patients were, the higher miR-21 expression level can be measured (Singh et al., 2018).

Oral leukoplakia (OLK) as the most common oral potentially malignant disorder (PMD) has a great connection with OSCC. The canceration rate of OLK is approximately 3%–5%. Chang et al. attempted to monitor the progress of OLK towards OSCC by the measurement of miRNAs to detect OSCC in early stages. They evaluated the expression of miR-222-3p, miR-423-5p and miR-150-5p in plasma from normal controls, OLK and OSCC patients. The expression was different among groups, and found that miR-222-3p and miR-423-5p were down-regulated when OSCC patients were in more advanced clinic stage, T stage and with lymph node metastasis status. They believed that the high diagnostic accuracy (AUC = 0.88) implied that the expression of these three miRNAs in plasma could be the potential biomarker for early detection of OSCC (Chang et al., 2018). The similar potential of monitoring OSCC in early stages also had been shown in miR-196a and miR-196b. Lu et al. compared the levels of circulating miR-196 among 53 healthy controls, 16 patients with oral precancer lesions and 90 patients with oral cancer. They demonstrated that circulating miR-196a and miR-196b were significantly up-regulated in patients with oral precancer lesions (5.9- and 14.8-fold, respectively) and patients with oral cancer (9.3- and 17.0-fold, respectively). And the combined detection of the levels of circulating miR-196a and miR-196b may be the promising biomarkers in the diagnosis of patients with oral precancer (AUC = 0.845) or oral cancer (AUC = 0.963), as well as in the monitoring of potential malignancy (AUC = 0.950, sensitivity = 91%, specificity = 85%) (Lu et al., 2015). Liu et al. found the discrimination between healthy controls and the OSCC patients by the level of circulating miR-196a (Liu et al., 2013).

According to studies that took samples in the pre-operative and post-operative patients, transcriptomics was also proved to have values in the monitoring of recurrence and prognosis of OSCC. In Yan et al. study, miR-486-5p plasmatic level was up-regulated in 16 of 20 (80%) post-operative patients compared to pre-operative patients; miR-486-5p plasmatic level was also significantly higher in healthy controls than pre-operative patients, while it was no significant difference between healthy controls and post-operative patients. This result suggested that miR-486-5p plasmatic level recovered after surgery. Besides miR-486-5p, Yan et al. found miR-375 and miR-92b-3p concentration

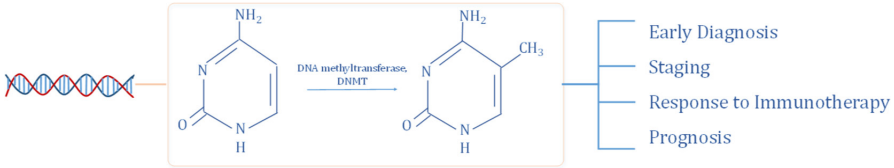

FIGURE 1 DNA methylation by liquid biopsy in OSCC

TABLE 3 Summary of liquid biopsy in saliva

Study cohort	Detection technique/platform	Molecular indicators	Omics
13 OSCC 10 HC	GoldenGate Methylation Array (Illumina)	DNA methylation	Genomics
45 OSCC 10 HC	qRT-PCR	miR-24-3p	Transcriptomics
23 OSCC 20 HC	ELISA	Alix expression	Proteomics
24 OSCC 10 HC	LC-MS/MS and label-free protein quantification (MaxQuant v.1.3.0.3)	Proteins	Proteomics
–	LC-MS/MS	Proteins	Proteomics
100 OSCC 50 OSMF 50 OLK 100 HC	ELISA	CYFRA 21-1	Proteomics
30OSCC 30 HC	UPLC-MS	Several bases; the combination of several amino acids	Metabolomics
37 OSCC 32 OLK 34 HC	Waters ACQUITY UPLC system (Waters Corporation, Milford MA)	The combination of valine, lactate, and phenylalanine	Metabolomics

Abbreviations: AUC, area under the curve; HC, healthy control; OLK, oral leukoplakia; OSCC, oral squamous carcinoma; OSMF, oral sub-mucous fibrosis.

in plasma was also significantly up-regulated in post-operative patients compared to pre-operative patients. The up-regulation of miR-486-5p、miR-375 and miR-92b-3p in the non-recurrence group showed a significant difference between pre-operative and post-operative samples, while in the recurrence group, the significant difference was not seen (Yan et al., 2017). Luo et al. showed the patients with a higher concentration of circ_0000199 (one of circular RNAs) sampling from circulating exosomes was at the risk of higher recurrence rate (HR, 3.36; 95% CI 2.12–5.26) and higher mortality rate (HR, 4.31; 95% CI 2.57–7.28), and it implied that the up-regulation of circulating exosomal circ_0000199 had a positive correlation with poor survival outcome (Luo et al., 2020). The significant down-regulation of miR-30a level in the serum of patients with OSCC was seen in the recurrence group with cisplatin treatment (Kulkarni et al., 2020).

4.3 | Proteomics

The protein reveals the genomic information and the development of the tumor. The analysis of proteomics can be useful in diagnosis, staging, and prognosis of OSCC. Oliveira-Costa et al. showed the possibility of PD-L1 as a biomarker of OSCC staging. They detected the expression of PD-L1 in CTCs, and found that the expression of PD-L1 was strong in cytoplasm of locally advanced OSCC patients (Oliveira-Costa et al., 2015). In the study contained 57 OSCC patients, serum samples and /or saliva samples were taken to detect the Alix expression level. They found that the Alix expression level was higher in OSCC patients than healthy controls, and the average serum Alix concentration was even higher in OSCC patients with advanced stages (Nakamichi et al., 2020). Winck et al. analyzed the proteome of whole saliva and salivary EVs in order to find out a new method to distinguish OSCC patients from the healthy. They discovered that there was 13 kinds of proteins were expressed in the significant difference between the “lesion” and “no lesion” group, while 38 kinds of proteins were only identified in saliva of patients with lesions. And 8 kinds of proteins in salivary EVs showed different expression level between patients and healthy controls (Winck et al., 2015). Wang et al. demonstrated the up-regulation of ZFAS1 expression in the serous exosomes of the patients who were resistant to cisplatin treatment. And the AUC of the ROC was 0.82. Considering above study also suggested that ZFAS1 promoted the resistance of cisplatin, it may become the therapeutic target in the future (Wang, Hao et al., 2020). Sivadasan et al. identified 1256 kinds of human proteins in saliva, and they got an updated human salivary proteome. 808 kinds of proteins of new human salivary proteome were reported because of their differential expression in oral cancer. Among them, 598 kinds of proteins of above 808 kinds of proteins had secretory features, and a subset of 139 kinds of proteins of them was more likely to be secreted. Thus this result provided new potential markers to discriminate OSCC patients (Sivadasan et al., 2015). C. Li et al. identified 415 kinds of proteins in serous exosomes. PF4V1 expression in whole blood was lower in OSCC patients with lymph node metastasis than healthy controls and OSCC patients without lymph node metastasis. F13A1 expression levels in whole blood was higher in OSCC patients compared to healthy controls (Li, Zhou, et al., 2019). The expression of lipopolysaccharide-binding protein (LBP) was up-regulated in serum of patients with OSCC compared to healthy controls, and the expression level of LBP would increase both in serum and serous exosomes in the patients with lymph node

Sensitivity/Specificity	AUC	Associations	Ref.
62% to 77%/83% to 100%	–	Early Detection	Viet & Schmidt (2008)
64.4%/80%	0.738	Diagnosis	He et al. (2020)
34.5%/100.0%	0.685	Staging	Nakamichi et al. (2020)
–	–	Diagnosis	Winck et al. (2015)
–	–	Diagnosis	Sivadasan et al. (2015)
75%/75%	0.895	Diagnosis and Early Screening	Rajkumar et al. (2015)
46.2% to 92.3%/ 61.5% to 96.7%; 100%/96.7%	0.708 to 0.994; 0.997	Diagnosis	Wang et al. (2014a, 2014b)
86.5% (OSCC-HC) and 94.6% (OSCC-OLK)/ 82.4% (OSCC-HC) and 84.4% (OSCC-OLK)	0.97 (OSCC-HC), 0.89 (OSCC-OL)	Diagnosis and Early Screening	Wei et al. (2011)

metastasis (Wang, Li, et al., 2020). Rajkumar et al. evaluated the level of CYFRA 21-1 (a constituent of the intermediate filament proteins of epithelial cells) in saliva and serum by enzyme-linked immunosorbent assay (ELISA). The results from healthy controls, patients with oral precancer and OSCC showed that the level of CYFRA 21-1 was significantly higher in OSCC patients, and it was also higher in patients with oral precancer than healthy controls. Another interesting thing was that they found that the level of CYFRA 21-1 in patients with OSCC was three-fold higher in saliva than serum, and therefore superior sensitivity was shown in saliva than serum in the detection of OSCC (Rajkumar et al., 2015).

4.4 | Metabolomics

The usual methods to analyze metabolomics are Nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), chromatography, and liquid chromatogram-mass spectrometry (LC-MS). Studies showed that the analysis of metabolomics may have feasibility for the early diagnosis and monitoring of OSCC. Wang et al. revealed a higher level of choline, betaine, and pipecolinic acid, and a lower level of l-carnitine in saliva of OSCC patients. They also identified 14 potential discriminant metabolites and suggested that the combination of propionylcholine, N-acetyl-l-phenylalanine, sphinganine, phytosphingosine, and S-carboxymethyl-l-cysteine could distinct OSCC patients from controls (Wang et al., 2014a, 2014b).

The difference of metabolites content among OSCC patients, healthy controls and OLK was also confirmed by Wei et al. In their study, dozens of kinds of metabolites concentration were significantly different in saliva between OSCC and healthy controls, OLK and healthy controls, and OSCC and OLK. They demonstrated the combination of valine, lactate, and phenylalanine had robust discrimination among OSCC, OLK and controls (Wei et al., 2011).

4.5 | DNA methylation

DNA methylation is one of the patterns of epigenetic regulation of chromatin. DNA methylation influences the structure, stability, and interaction with proteins. It finally has an impact on DNA expression. The different levels of DNA methylation lead to the different performances of cells. In some loci, the level of DNA methylation between oncologic patients and healthy groups was significant. DNA methylation is stable enough so that they can be detected in liquid biopsy. The detection of DNA methylation always includes a set of gene loci to promote accuracy. Because DNA methylation is the upstream of gene regulation, the detection of DNA methylation have a greater advantage in cancers' early screening.

Lots of reported have published on the detection of DNA methylation in various cancers. Chen et al. detect methylation level in ctDNA, and showed that the specific methylation signals could be detected up to four years before common standard detection in stomach, esophageal, colorectal, lung or liver cancer patients (Chen et al., 2020). According to Galle et al. study, the changes of methylation of ctDNA could be the indicators to predict the response to sorafenib in HCC patients (Galle et al., 2020). The methylation status of insulin-like growth factor binding protein 7 (IGFBP7) promoter was an independent risk factor of overall survival (OS) early tumour recurrence (ETR) in HCC patients (Li et al., 2018). Downs et al. found 8 kinds of methylation markers which could distinguished DLBCL

from gliomas. They further invented a method using 2 kinds of methylation markers (cg0504 and SCG3 triplexed with ACTB) to discriminate primary central nervous system lymphoma (PCNSL) from other central nervous system (CNS) tumors (Downs et al., 2021). Park et al. detected the methylation level of *LINE-1* in cfDNA, and demonstrated that *LINE-1* methylation levels were significantly lower in lung cancer and breast cancer patients than healthy controls (Park et al., 2021). Zhang et al. evaluated the methylation levels of cfDNA, and found that the methylation levels of c9orf50, twist1, kcnj12, and znf132 were higher in colorectal cancer (CRC) patients. The combination of above four gene methylation levels could assist in staging of CRC patients (Zhang et al., 2021).

In Viet et al. study, they analyzed the levels of DNA methylation of 807 cancer-associated genes in saliva of 13 OSCC patients and 10 healthy controls. According to their results, they identified 41 gene loci that were in high level of DNA methylation in preoperation patients while were not methylated in healthy controls and post-operative patients. They constructed gene panels and showed that the analysis of methylation in saliva could be used in the early detection of OSCC (Viet & Schmidt, 2008). (Figure 1).

4.6 | Multiomics analysis

The results mentioned above were involved in single-omic analysis. Further, integrating multiomics analysis in liquid biopsy also can be used in OSCC. Multiomics analysis can reveal information from different levels, and therefore improve the accuracy of the analysis. According to the result of qPCR, F13A1 mRNA expression level was significantly higher in whole blood of patients with OSCC than healthy controls. The up-regulation of F13A1 expression was shown in patients who had positive cervical lymph node, compared to healthy controls and patients without metastasis (Liu, 2016).

B. Li et al thought that some patients' hypercoagulable state caused by OSCC was the result of general inflammatory state and the release of neutrophil extracellular traps (NETs), which were consisted of DNA and antimicrobial peptides. They detected above components (cfDNA, myeloperoxidase-deoxyribonucleic acid [MPO-DNA] complex, nucleosomes, and neutrophil elastase [NE]) concentration to be the replacement of the concentration of NETs. NE, cfDNA, MPO-DNA complex serous level in clinical stage III/IV patients was higher than healthy controls and clinical stage I/II patients. These differences implied that one of the possible ways to eliminate the hypercoagulable state in stage III/IV patients is to block the release of NETs (Li, Liu, et al., 2019). (Figure 2).

5 | CURRENT STATUS AND PROSPECT

The studies of liquid biopsy in OSCC patients are fewer than some other tumors. And the studies were based on some different OSCC issues, such as early screening, diagnosis, staging, monitoring of recurrence, the judgment of the treatment and prognosis. The results of the studies were not very suitable to be merged. So we choose to do a narrative review rather than a systematic review.

All in all, the analysis of multiomics of liquid biopsy in oral cancer represents a promising opportunity for the assist of early screening, diagnosis, staging, monitoring of recurrence, the judgment of the treatment and prognosis. Especially, the analysis of omics of liquid biopsy in oral cancer may bring out promotion in the early detection rate of OSCC, and that may be a breakthrough for improving the survival rate of patients with OSCC. Based on available studies, the analysis of genomics of liquid biopsy in OSCC focused on cfDNA. The potential was shown mainly in the prediction of metastasis and prognosis. The researchers paid great attention and effort on miRNA in the analysis of transcriptomics, and the characteristics of miRNA

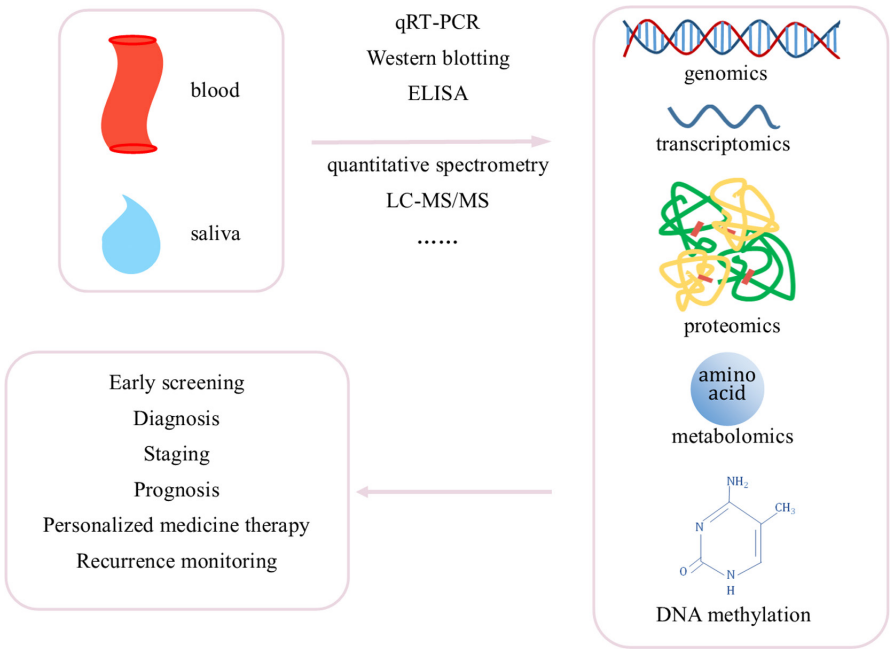

FIGURE 2 Multiomics analysis of liquid biopsy in OSCC

that can regulate gene expression and make them show great potential in early screening, diagnosis, staging, monitoring of recurrence, and prognosis of OSCC. The analysis of proteomics was often sampled from exosomal proteins in saliva and blood, and could be helpful in the assistance of diagnosis and personalized treatments of OSCC. The levels of proteins or their constituent in saliva may be the promising biomarkers for early screening. As for the analysis of metabolomics, researchers usually sampled from saliva because of its tight relationship with oral cancer, and the combination of different metabolites showed feasibility in the diagnosis and monitoring of OSCC.

5.1 | Advantages

As mentioned above, liquid biopsy can provide information about OSCC in real time and repeatedly. So liquid biopsy is a benefit to establish the dynamic monitoring of OSCC. And it can help us understand the changes of OSCC in spatial and temporal aspects. It is the base of precision medicine. Besides, liquid biopsy is convenient and minimally invasive for patients. It reduces the risk of surgery. Liquid biopsy also has the potential to detect MRD, which is impossible for tissue biopsy. Because liquid biopsy can be carried out at the molecular level, it may diagnose OSCC earlier. Also, the application of liquid biopsy may improve the rates of survival rate.

5.2 | Disadvantages

There are still several problems that remained to be solved. (1) The lackness of the standard of the measurement methods and procedures of samples. Reviewing the published paper, the measurement methods and procedures of samples were different between studies, such as western blotting, ELISA, and immunohistochemistry of proteomics, NMR, MS, chromatography, and LC-MS of metabolomics. The difference of the measurement methods and procedures reduces the comparability among them. It also brings out difficulties when we would like to integrate the results of different studies. (2) Various measurement methods and procedures may not accomplish at the same time in limited samples. It will cause the prolongation of detection time and the waste of specimens. In recurrent reported studies, except some studies of proteomics and metabolomics, many studies just involved indicators of a dimension. Due to the difficulty in integrating of results we aforementioned, many results were hard to be used in the analysis of omics. (3) The conjoint analysis of multiomics is not enough, and the reports don't show too much connection among different omics. At the same time, the sensitivity and the specificity of some studies' results still did not meet the criteria for clinical application.

5.3 | Future perspectives

The analysis of multiomics of liquid biopsy in oral cancer shows its capacity in clinical application. We should establish the standard of measurement methods and procedures of samples in the next step to increase the comparability of different results and get more effective indicators. More efforts should be made on searching molecular indicators with higher sensitivity and specificity. For example, apply single cell technique and spatial transcriptome technique to liquid biopsy. And the sample size should be expanded in future studies. Considering that we will need to handle plenty of data if the multiomics analysis is applied in clinic, we may established a model to predict patients condition by using artificial intelligence (Yang et al., 2020).

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CONFLICTS OF INTEREST

The author declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

AUTHOR CONTRIBUTIONS

Dr. Binbin Li was involved in the conception and design of the study, drafted and revised the manuscript, and provided final approval of the version to be published. Xinning Zhang drafted and revised the manuscript, and provided final approval of the version to be published.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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